

Principal Diseases of Marine Fish and Shellfish

Volume 2
Second Edition



. . .

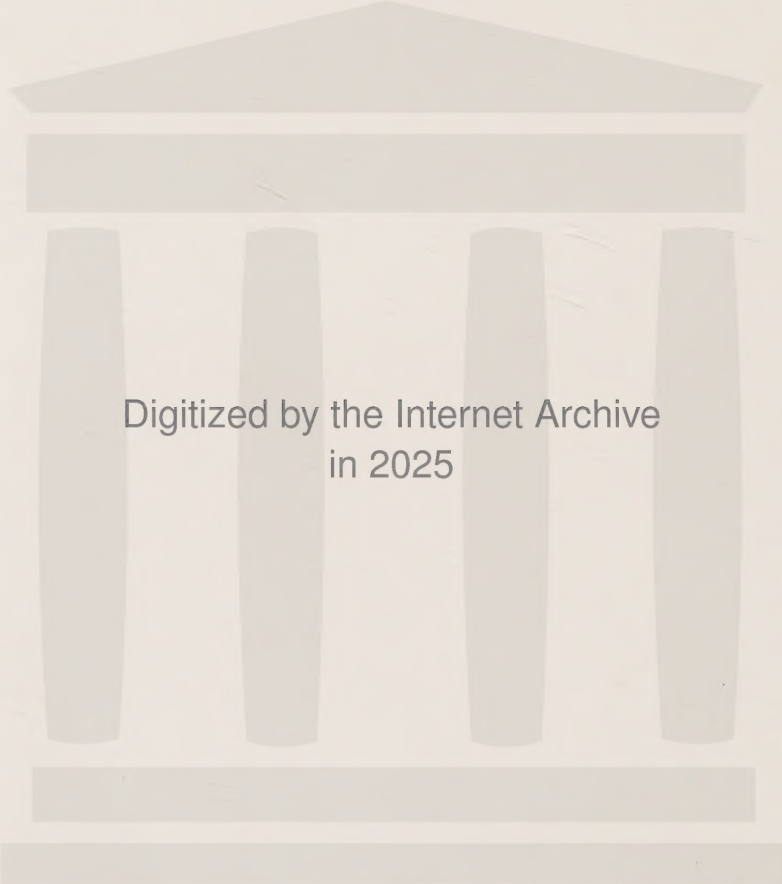
Carl J. Sindermann

NMFS Honolulu Library



00000000002961

Library
NOAA, National Marine Fisheries Service
SWFC Honolulu Laboratory F/SWC2
2570 Dole Street
Honolulu, HI 96822-2396



Digitized by the Internet Archive
in 2025

Principal Diseases of Marine Fish and Shellfish

Writers need understanding, support, and even love, if they are to perform most effectively. My best source of these vital elements has been my wife, Joan, to whom this book is dedicated.

Principal Diseases of Marine Fish and Shellfish

Second Edition

Volume 2

Diseases of Marine Shellfish

Carl J. Sindermann

Oxford Laboratory

Northeast Fisheries Center

National Marine Fisheries Service

National Oceanic and Atmospheric Administration

Oxford, Maryland

SH
171
1553
1989
V.2



Academic Press, Inc.

Harcourt Brace Jovanovich, Publishers

San Diego New York Boston London Sydney Tokyo Toronto

This book is printed on acid-free paper. (∞)

Copyright © 1990, 1970 by Academic Press, Inc.

All Rights Reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Academic Press, Inc.
San Diego, California 92101

United Kingdom Edition published by
Academic Press Limited
24-28 Oval Road, London NW1 7DX

Library of Congress Cataloging-in-Publication Data

(Revised for volume 2)

Sindermann, Carl J.

Principal diseases of marine fish and shellfish.

Includes bibliographies and index.

Contents: v. 1. Diseases of marine fish -- v. 2.

Diseases of marine shellfish.

1. Marine fishes--Diseases. 2. Shellfish--Diseases.

I. Title

SH171.S53 1989 639.3 89-30282

ISBN 0-12-645851-0 (v. 1 : alk. paper)

ISBN 0-12-645852-9 (v. 2 : alk. paper)

Printed in the United States of America

90 91 92 93 9 8 7 6 5 4 3 2 1

Contents

Contents of Volume 1	ix
Preface	xi

Part I. Introduction

1. An Overview

Part II. Diseases of Shellfish Caused by Microbial Pathogens and Animal Parasites

2. Viruses

I. Introduction	9
II. Viral Diseases of Molluscs	10
III. Viral Diseases of Crustaceans	20
IV. Conclusions	34
References	34

3. Bacteria

I. Introduction	41
II. Bacterial Diseases of Molluscs	41
III. Bacterial Diseases of Crustaceans	48
IV. Conclusions	60
V. Addendum: Chlamydial and Rickettsial Infections of Molluscs and Crustaceans	60
References	62

4. Fungi

I. Introduction	71
II. Molluscan Fungal Diseases	71
III. Crustacean Fungal Diseases	76
IV. Conclusions	85
References	86

5. Protozoans

I. Introduction	93
II. Protozoan Diseases of Molluscs	93

III. Protozoan Diseases of Crustaceans	117
IV. Conclusions	130
References	131
6. Helminths	
I. Introduction	145
II. Diseases of Molluscs Caused by Helminths	145
III. Diseases of Crustaceans Caused by Helminths	162
IV. Conclusions	169
References	170
7. Parasitic Crustaceans	
I. Introduction	183
II. Diseases of Molluscs Caused by Parasitic Crustaceans	183
III. Diseases of Crustaceans Caused by Parasitic Crustaceans	190
IV. Conclusions	197
References	198
Part III. Noninfectious Diseases of Shellfish	
8. Neoplastic Diseases	
I. Introduction	209
II. Neoplastic Diseases of Molluscs	211
III. Neoplastic Diseases of Crustaceans	226
IV. Conclusions	228
References	229
9. Genetic and Environmentally Induced Abnormalities	
I. Introduction	235
II. Molluscan Abnormalities	236
III. Crustacean Abnormalities	239
IV. Conclusions	241
References	242
Part IV. Responses of Shellfish to Pathogens	
10. Mechanisms of Internal Defense	
I. Introduction	247
II. Mechanisms of Internal Defense in Molluscs	249
III. Mechanisms of Internal Defense in Crustaceans	267
IV. Conclusions	288
References	290
Part V. Diseases of Captive Shellfish	
11. Disease in Impounded Populations	
I. Introduction	303
II. Types of Disease Conditions Seen in Impounded Populations	304

III. Disease Control Methods in Impounded Populations	308
IV. Conclusions	309
References	309

12. Disease in Shellfish Culture

I. Introduction	311
II. "Case Histories" of the Effects of Disease on Shellfish Culture	315
III. Assessment of Disease Effects on Cultured Shellfish	321
IV. Principles and Mechanisms of Disease Control in Shellfish Culture	326
V. Conclusions: Emerging Concepts in Shellfish Pathology	348
References	350

Part VI. Disease as a Causal Factor in Mass Mortalities of Shellfish

13. Epizootics and Mass Mortalities of Shellfish

I. Introduction	359
II. Epizootics and Mass Mortalities of Molluscs	361
III. Epizootics and Mass Mortalities of Crustaceans	388
IV. Conclusions	397
References	399

Part VII. Shellfish Diseases and Humans

14. Pollution-Associated Diseases

I. Introduction	413
II. Diseases Caused by Contaminant Stress and Facultative Pathogens	415
III. Stress-Provoked Latent Infections	422
IV. Genetic and Environmentally Induced Abnormalities	423
V. Experimentally Induced Lesions	427
VI. Contaminant Effects on Disease Resistance	433
VII. Conclusions	434
References	436

15. Economic Effects of Shellfish Diseases

I. Introduction	443
II. Effects of Disease on Producers and Consumers	444
III. Losses in Shellfish Aquaculture	447
IV. Losses in Natural Shellfish Populations	450
V. Conclusions	452
References	453

16. Shellfish Diseases of Public Health Significance

I. Introduction	457
II. Shellfish Pathogens with Demonstrated Public Health Significance	458
III. Shellfish Pathogens with Less Clearly Defined Public Health Significance	466
IV. Diseases of Humans Transmitted Passively by Shellfish	468

V. Conclusions	480
References	481
17. Conclusions	
I. Disease Resistance in Shellfish	489
II. Characteristics of Epizootics in Shellfish	490
III. Disease Control in Shellfish Populations	491
IV. Diagnostic Methods	493
V. Risks of Disease from Transfers and Introductions	493
References	495
Epilogue	497
Index	501

Contents of Volume 1

Part I. Introduction

1. Terms of Reference

Part II. Diseases of Marine Fish Caused by Microbial Pathogens and Animal Parasites

2. Viruses
3. Bacteria
4. Fungi
5. Protozoans
6. Helminths
7. Parasitic Crustaceans

Part III. Noninfectious Diseases of Marine Fish

8. Neoplastic Diseases
9. Genetic and Environmentally Induced Abnormalities

Part IV. Responses of Marine Fish to Pathogens

10. Mechanisms of Internal Defense

Part V. Diseases of Captive Marine Fish

11. Disease and Parasite Problems in Marine Aquaria
12. Disease and Parasite Problems in Marine Fish Culture

Part VI. The Role of Disease in Marine Fish Populations

13. The Role of Disease in Marine Fish Populations: An Overview
14. Effects of Microbial Diseases on Marine Fish Populations
15. Effects of Animal Parasites on Marine Fish Populations
16. Mass Mortalities of Marine Fish

Part VII. Marine Fish Diseases and Humans

17. Pollution-Associated Diseases of Marine Fish

- 18. Economic Effects of Marine Fish Diseases
- 19. Marine Fish Diseases of Public Health Significance

Part VIII. Diseases of Marine Fish: Concepts, Control, and Future Research

- 20. Conclusions

Preface

The first edition of *Principal Diseases of Marine Fish and Shellfish* was published twenty years ago (1970), as an attempt to summarize a rapidly expanding (for those ancient times) body of literature and to examine the developing conceptual base for marine fish and shellfish pathology. A revision—or preferably a complete rewriting—has been long overdue in view of the rate of accumulation of new information. This work, now in two volumes, is a response to a perceived need for a current assessment of the status of knowledge about marine fish and shellfish diseases. It tries to blend the old and the new, especially since much fundamental work in fish pathology was done before the average reader was born. (This may not be true for shellfish pathology, which is a more recent development.) An abundance of new data has been integrated here with results of earlier studies, as an illustration of the continuum that is the essence of scientific research.

I have many people to thank for the (reasonably) timely completion of Volume 2 of *Principal Diseases of Marine Fish and Shellfish*. Since the original drafts were handwritten, I have depended on the word processing and editing skills of Mrs. Kathe Melkers of the Sandy Hook (New Jersey) Laboratory and Mrs. Jane Swann of the Oxford (Maryland) Laboratory to transform very rough copy into documents of great precision. I have also depended on Mrs. Michele Cox of the Sandy Hook Laboratory to produce illustrations that have added substantially to the utility of the volume.

Volume 2 was completed during an Intergovernmental Personnel Act (IPA) appointment with the Maryland Department of Natural Resources at the Oxford Cooperative Research Laboratory, Oxford, Maryland. I want to thank Dr. George E. Krantz, Director of the Oxford Laboratory, and Mr. Allen E. Peterson, Jr., Director for Science and Research, Northeast Region, NMFS, for administrative support and encouragement during the course of the IPA appointment.

I also want to express appreciation for helpful comments from reviewers of some of the draft chapters, particularly Dr. Phyllis Johnson,

formerly of the Oxford Laboratory, Mr. C. Austin Farley of the Oxford Laboratory, Dr. Richard Robohm of the Milford (Connecticut) Laboratory, and Dr. Joanne Stolen of the Sand Hook Laboratory. Their comments have been valuable in improving some presentations of information, but of course ultimate responsibility for statements of fact or opinion rests with me.

I have been absolutely astounded by the amount of new literature on shellfish diseases published in the past two decades. Information expansion has followed a logarithmic growth curve, so that any summarizer or interpreter of that flood of new data soon feels (on some weekday mornings, at least) like the classic sorcerer's apprentice. Slowly, though, the peaks of new information and new insights break through the mist, and the boundary between knowledge and ignorance is seen to have receded, however slightly, during the night. I hope that these volumes will stimulate increased interest in and investigation of the role of diseases in fish and shellfish populations, and that they will be useful to marine scientists in examining the status of knowledge in a fascinating and very significant aspect of marine biology.

Carl J. Sindermann

Part I

Introduction

1

An Overview

As understanding of factors that influence the numbers of animals in the sea has expanded, it has become evident that disease, among other environmental variables, can drastically affect abundance. This fact has been clearly demonstrated in several populations of inshore invertebrates, many of which are harvested in great numbers and constitute marine crops of high value. Some, such as mussels, oysters, clams, crabs, lobsters, and shrimp, occur in inshore or estuarine waters and have been subjected to varying degrees of cultivation in different parts of the world. Under natural conditions or cultivation, mass mortalities occasionally occur; here disease can be an important contributing factor.

Much of our knowledge about diseases of marine invertebrates concerns species of economic importance, particularly the crustacean and bivalve molluscan shellfish. Major contributions to the shellfish disease literature have been made by North American, Western European, and Japanese scientists. This volume clearly has a North American bias, but some attempts have been made to recognize work done elsewhere in the world as well. There is little representation of the Russian literature here; this may be, in part, a reflection of the relative lack of emphasis placed on shellfish in Russian fisheries research, as well as the limited availability of translations of Russian literature. The Japanese literature is undoubtedly underrepresented, also because of the translation problem (although English abstracts now accompany many Japanese papers).

A number of very helpful reviews and books have appeared within the past decade, including the massive four-volume *Diseases of Marine Animals*, edited by Otto Kinne, *Synopsis of Invertebrate Pathology*, written by Albert K. Sparks, and *Histology of the Blue Crab*, written by Phyllis T. Johnson. Useful reviews of selected aspects of shellfish diseases include those on lobsters, by James E. Stewart (1975, 1980, 1984), and on crustacean microbial diseases, by Phyllis T. Johnson (1983, 1984) and by John A. Couch (1978, 1981).

The objectives of this volume on shellfish diseases are

- To summarize available information about diseases of commercially important molluscs and crustaceans
- To focus on pathogens and parasites that severely damage host animals, and to give only minimal attention to organisms that do not cause obvious pathology
- To consider in detail the internal defense mechanisms of shellfish—insofar as present understanding will allow
- To examine in some depth the role of disease in shellfish culture
- To assess the effects of disease on shellfish populations—especially the effects of epizootics
- To look at evidence for effects of environmental degradation on shellfish
- To summarize information on economic effects of shellfish diseases
- To review the associations of shellfish diseases and public health

The structure of Volume 2 follows what seems to me a logical progression—beginning with a systematic consideration of diseases caused by different major groups of pathogens, from viruses to parasitic Crustacea, progressing to the so-called “noninfectious” diseases, and moving then to the special topics of disease resistance and disease in captive populations. These parts are followed by an examination of the effects of epizootic diseases on shellfish populations, and the volume concludes with an examination of the principal ways in which shellfish and human populations interact. This structure has provided an apparently workable sequence of major topic areas for this volume, and it closely parallels the structure chosen earlier for Volume 1 on fish diseases.

One problem, identified early, is that this structure and approach may lead to just a soupçon of redundancy. My immediate rationalization is that maybe a little repetition is in order, since readers of the book will undoubtedly be selective, consulting only those chapters of immediate interest to them and ignoring (or, at best, scanning) the rest. Exceptions to this dismal prediction might be a few persistent, dedicated reviewers, who may read the entire book; otherwise, the chapters will probably be treated much like the ingredients of a smorgasbord. If this assumption is valid, then each chapter has to be largely free-standing, without too much frustrating reference to other chapters. The independence of chapters is relative, of course, because they form parts of larger units (the sections), and such units, when suitably integrated, can provide a reasonable framework for discussion of the entire subject of shellfish diseases.

One obvious structural anomaly in Volume 2 is that the chapters differ markedly in length. To some extent this disparity evolved naturally, based on relative amounts of literature available; to a larger extent, though, the chapter lengths reflect the relative impacts on shellfish populations (in Parts II and III, on systematic treatments of infectious and noninfectious diseases) and the relative emphasis of current research and the status of knowledge (in Parts IV through VII, on the subjects of disease resistance, disease in captive populations, assessment of disease effects, and shellfish diseases and humans).

It might be good at the outset to confront any vague uneasiness about use of the term "shellfish" in this volume. I have elected to use it in its broadest definition, as including both molluscan and crustacean species. Some authors limit the term to molluscan species only, while others broaden it to include crustaceans as well, in the sense that they are all "shelled" animals (with a few exceptions among the molluscs). My choice is the broader definition, particularly since this book has a practical orientation, emphasizing diseases of species with commercial significance. I will admit, however, that assembling economically important representatives of Mollusca and Crustacea in chapters labeled "shellfish" is somewhat artificial, since these groups are evolutionarily very divergent—so universal conclusions should not be expected (as was pointed out in another context by Warr, 1981).¹

While in the mood for definitions, it might also be good to circumscribe the term "disease"—again in its broadest sense, as "any departure from normal structure or function of the animal." Infectious diseases (those caused by microbial pathogens or animal parasites) are of greatest importance and will receive greatest attention here, but the noninfectious diseases (genetic and environmentally induced abnormalities) will not be ignored completely.

¹Warr, G. W. (1981). Immunity in invertebrates. *J. Invertebr. Pathol.* **38**, 311–314.

Part II

Diseases of Shellfish Caused by Microbial Pathogens and Animal Parasites—Infectious Diseases

Infectious diseases of molluscan and crustacean shellfish come in a bewildering array, so it is entirely logical to impose an artificial structure on any treatment of the topic. One approach is to consider pathogens in systematic progression—viruses, bacteria, fungi, protozoans, helminths, and parasitic crustaceans—and to include separate treatments of molluscan and crustacean pathogens in each of these six groups. This plan has been followed in creating the chapters included in this section, and it does seem to result in useful pigeonholes for discussion purposes. The structure is as follows:

Chapter 2: Viruses

Chapter 3: Bacteria

Chapter 4: Fungi

Chapter 5: Protozoans

Chapter 6: Helminths

Chapter 7: Parasitic Crustaceans

The chapters are of unequal lengths; some are markedly longer than others. This should reflect (1) the amount of information available and (2) the relative importance of the group as pathogens of shellfish. There may be a touch of subjectivity to some of the “assessments of worth” here, but the volume is meant to be more than a literature review, so some gentle expressions of opinion should not be too far out of line.

2

Viruses

I. INTRODUCTION

Knowledge of viral diseases of crustacean and molluscan shellfish is all of recent origin—first reports date back only to the mid-1960s for crustaceans and to the early 1970s for molluscs. At present more than 30 viruses have been described, in varying detail, from marine crustaceans and about 20 from marine molluscs.

Progress in understanding the role of viral infections in shellfish has thus been substantial, but it has been severely limited by the lack of stable cell culture systems of molluscan or crustacean origin. None of the shellfish viruses has been adequately characterized; usually, only information on histopathology and ultrastructural morphology is available. A few viruses have been associated with pathological changes in the host, and a few have been transmitted experimentally; many have not yet been definitely associated with a specific disease condition.

With the present knowledge reasonable statements are that some viruses seem clearly pathogenic to shellfish hosts, many more exist in latent form in their hosts, and some are probably contaminants which may be isolated from shellfish but do not cause infections. Furthermore, it seems that, at least in Crustacea, some viruses act synergistically, and multiple infections exist.

A problem that has arisen repeatedly in descriptions of presumptive viral diseases of shellfish is *the demonstration of a causal relationship between the virus and the pathology seen*. Usually, the agent has not been isolated, nor characterized biochemically or serologically, nor has the disease been reproduced experimentally (except in a few instances), so Rivers' postulate² has not been satisfied in most instances. Diagnosis is based

²Rivers' postulate requires the etiological agent to be isolated from a diseased host and passed via a cell-free extract to a susceptible host in which the same disease is later manifested. It must be demonstrated that a virus is not only associated with a disease but

on ultrastructural studies and the occurrence of typical lesions; the possible role of other agents in producing the disease has not been excluded.

Despite these limitations, a body of literature on viral diseases of shellfish is developing. Some of it is summarized here, with a clear appreciation for the rapid movement of research on the topic.

II. VIRAL DISEASES OF MOLLUSCS

Viruses were unknown in marine molluscs until the early 1970s. In 1971 two reports were published on viral pathogens of cephalopods—a presumptive iridovirus in *Octopus vulgaris* by Rungger *et al.* (1971) and another in *Sepia officinalis* by Devauchelle and Vago (1971). The first report of viruses in commercial bivalves was that of Farley *et al.* (1972) on a herpes-type infection of oysters (*Crassostrea virginica*). The years since then have seen expansion of the literature on molluscan viruses, enhanced appreciably by contributions of French workers examining causes of extensive recent oyster mortalities, some of which seem to have a viral etiology. Reviewing available reports, as well as summarizations by Farley (1978, 1981), Lauckner (1983), and Sparks (1985), it is apparent that:

1. Few of the viruses seen in marine invertebrates have been associated conclusively with mortality, or even with pathology, and experimental transmission has rarely been accomplished (none of the iridoviruses, for example, has been transmitted in this way). Effects on survival are usually undetermined, in part because of a lack of suitable experimental systems.
2. None of the molluscan viruses has been adequately characterized; often, only information on histopathology or ultrastructural morphology is presented, since standardized molluscan cell cultures are not available.
3. Published literature on marine invertebrate viruses in general is still sparse; however, its rate of accumulation during the past decade suggests that viral infections are common, but as yet unrecognized. Farley (1981) has further pointed out that "All

is actually the cause. In the words of Rivers (1937), ". . . this is accomplished by the production with a degree of regularity of a transmissible infection in susceptible experimental hosts by means of inoculation of material, free from ordinary microbes or rickettsiae, obtained from patients with the natural disease, and by the demonstration through the use of proper controls and immunological studies described above that the virus was neither fortuitously present in the patients nor accidentally picked up in the experimental animals."

viruses from marine invertebrates can be placed provisionally in families of viruses found in vertebrates, with the exception of *Baculovirus* which apparently occurs only in arthropods," that "... most invertebrate virus families are represented in the marine invertebrates . . .," and that "... no new major taxa [of viruses] have been found in marine invertebrates, even though over 30 invertebrate viruses have been described."

4. Some viruses have been detected in marine invertebrates affected by other diseases or under some form of environmental stress, so it is uncertain whether the viruses are primary pathogens or secondary invaders. An example of this can be seen in descriptions of a disease affecting the velar epithelium of oyster (*Crassostrea gigas*) larvae. A hexagonal virus seems associated with velar erosion, but etiology is confused by the occurrence of funguslike organisms which seem responsible for gastrointestinal impactions (Elston, 1979, 1980; Leibovitz *et al.*, 1978) in the same animals, although a later report (Elston and Wilkinson, 1985) distinguished specific viral effects on the velum.
5. A number of recent reports (such as that on herpes-type viral infections of oysters) suggest that the viral agents are latent in natural populations but may become patent under conditions of environmental stress (such as those found in polluted waters or in aquaculture facilities).

Beyond these general observations, it would seem appropriate to examine some examples of viral diseases (often presumptive) of economically important molluscs. Diseases which have received scientific attention are summarized in Table 1; these have been aggregated by host species, even though the degree of host specificity is unknown for most of the viral agents discussed.

Oysters, because of their overriding economic importance worldwide, have been examined for diseases which affect survival. Although certain protozoans (to be considered in Chapter 5) seem to be predominant pathogens of oysters, viral diseases are also known. Of these, epizootics caused by several iridoviruses are probably most significant. The first outbreaks occurred in Portuguese oysters, *Crassostrea angulata*, on the French coast beginning in 1966 and peaking in 1967 (Marteil, 1971). Known as *maladie des branchies*, or gill disease, the outbreaks, with mortalities up to 70% in some culture areas, were attributed initially to several protistan pathogens, but a viral (iridoviral) etiology was proposed in 1976 (Comps and Duthoit, 1976). Then, beginning in 1970, new mortalities of *C. angulata*, caused by another (and similar) iridovirus,

Table 1. Viruses from Marine Mollusca

Host	Virus Type	Location in Host and Pathology	Effect on Host	Geographic Distribution	References
<i>Ostrea edulis</i> (European oyster)	IPN-like reovirus (originally isolated from <i>Tellina tenuis</i> as well as <i>O. edulis</i> and called "Tellina virus")	Digestive gland	Experimental infections caused necrosis of digestive tissue, with infiltration of hemocytes; general tissue edema; increased mortality rates in experimental populations	United Kingdom	Hill (1976a,b), Hill and Alderman (1979), Underwood <i>et al.</i> (1977)
<i>Crassostrea virginica</i> (American oyster)	Herpes-type	Intranuclear, with Cowdry type A inclusions in cells surrounding hemolymph sinuses. Note: other Cowdry type A intranuclear inclusions have been seen in mantle epithelium of juvenile oysters (Meyers, 1981)	Mortalities when animals stressed by high temperatures. Dilated digestive diverticula, and aggregation of cells in connective tissue surrounding blood sinuses	Piscataqua River, Maine	Farley <i>et al.</i> (1972)
	Papovavirus (similar to papillomavirus)	"Ovacystis" disease—massive hypertrophy of gametocytes and eggs; nuclei contain large Feulgen-positive viral masses. Note: similar lesions have been seen in other bivalves	Lysis of infected cells; occurs in low prevalence	Chesapeake Bay; Long Island Sound; Maine	Farley (1973, 1975, 1976a,b, 1978), Meyers (1981)

Reo-like virus	Isolated from tissue homogenates of two juvenile oysters in samples obtained for viral screening	Isolates were cytopathogenic for fish cell lines, but no oyster pathology was reported	Molluscan hatchery, Long Island, New York	Meyers (1979), Meyers and Hirai (1980)
<i>Crassostrea gigas</i> (Pacific oyster)	Herpes-type (presumptive)	Intranuclear with Cowdry type A inclusions	Associated with enzootic "summer disease" of <i>C. gigas</i>	Found in oysters imported to North Wales for cultivation
	Icosahedral virus similar to that seen in gill disease of <i>Crassostrea angulata</i> in France; probably an iridovirus	Velar epithelial cells of larvae, with intracytoplasmic inclusions; rare in mantle epithelial cells	Velar and mantle erosion; epizootic with mortalities of larvae up to 50%; called oyster velar virus disease (OVVD)	Molluscan hatchery on Pacific coast of the U.S. (State of Washington)
	Iridovirus	Gill and palp tissues	Gill erosions, similar to those seen in <i>maladie des branchies</i> of <i>C. angulata</i> , but of lesser severity than in that species	Molluscan hatchery on Pacific coast of the U.S. (State of Washington)
			Seen in <i>C. gigas</i> transplanted to French waters from Japan, Korea, and Canada during 1966–1969 but not subsequently	Elston (1979, 1980), Leibovitz <i>et al.</i> (1978), Elston and Wilkinson (1985)
			Marteil (1968, 1969), Comps (1970, 1972)	
	Icosahedral virus (presumptive) resembling iridovirus of <i>C. angulata</i>	Connective tissue cells	Causes grayish discoloration of visceral mass	Seen in depuration plant at Arcachon, where it caused mortalities of adult oysters; also seen in cultivated oysters from the
			Seen in depuration plant at Arcachon, where it caused mortalities of adult oysters; also seen in cultivated oysters from the	Comps (1978, 1980, 1983), Comps and Bonami (1977), Comps and Duthoit (1979)

(continued)

Table 1. (Continued)

Host	Virus Type	Location in Host and Pathology	Effect on Host	Geographic Distribution	References
<i>Crassostrea angulata</i> (Portuguese oyster)	Iridovirus, resembling lymphocystis virus of fish	Gill and palp tissues, leading to destruction of filaments	Identified as cause of <i>maladie des branchies</i> , resulting in mass mortalities on French Atlantic coast 1966–1969, persisting at enzootic levels since then in surviving populations; causes gill erosion and necrosis (but experimental transmission not accomplished); characteristic histopathology includes hypertrophy of gill epithelial cells	Bay of Arcachon and other growing areas on the French coast France, Spain, Portugal	Deltreil (1969, 1971), Franc and Arvy, (1969, 1970), Comps (1969, 1970, 1972, 1978), Arvy and Franc (1968), Besse (1968), Martell (1968, 1969), Alderman and Gras (1969), Gras (1969), Comps and Duthoit (1976, 1979), His (1969)
	Iridovirus-like (presumptive)	Connective tissue cells	Thought to be cause of mass mortalities in 1970–1973, but experimental transmission not reported; resulted in drastic reduction of <i>C. angulata</i> population by 1973	Atlantic coast of France	Comps <i>et al.</i> (1976), Comps (1978), Martell (1976), Bonami (1977)

<i>Mercenaria mercenaria</i> (hard-shell clam)	Herpes-type	Herpes-type intranuclear inclusions seen in cells of gonadal tumors	Role in neoplasia not determined	Northern New England	Barry and Yevich (1972), Farley (1978)
<i>Mya arenaria</i> (soft-shell clam)	B-type retrovirus (presumptive)	Isolated from homogenates	Associated with disease known as hemocytic sarcoma in clams (disease reproduced experimentally from filtrates of infected clams)	Atlantic coast of U.S.	Oprandy <i>et al.</i> (1981), Cooper <i>et al.</i> (1982a,b), Appeldoorn <i>et al.</i> (1979)
	Papovavirus resembling polyomavirus (presumptive)	Connective tissue, hemocytes, gill epithelium	May cause cell hypertrophy; intranuclear inclusions seen in hemocytes	Atlantic coast of U.S.	Rosenfield (1976), Farley (1976a,b, 1977, 1978), Harshbarger <i>et al.</i> (1979)
	Papillomavirus (presumptive)	Ciliated epithelia associated with food grooves of gills	Damage to gill tissues in form of hyperplasia and inflammation	Upper New York Bay	Koepp (1984)
<i>Octopus vulgaris</i> (octopus)	Iridovirus (presumptive)	Muscle cells	Causes intramuscular lesions, with edematous nodules on body surfaces, first appearing on the tentacles	Seawater tanks of Zoological Station at Naples, Italy; wild populations of Bay of Naples	Rungger <i>et al.</i> (1971)
<i>Sepia officinalis</i> (cuttlefish)	Reovirus (presumptive)	Stomach epithelial cells; often forms intracytoplasmic paracrystalline arrays	Cell lysis	France	Devauchelle and Vago (1971)

reduced the oyster population so severely that a replacement species, *C. gigas* from Pacific waters, was introduced in large quantities to ensure the survival of oyster culture in France (Comps *et al.*, 1976; Marteil, 1976; Bonami, 1977; Comps, 1978). *Crassostrea gigas* was partially resistant to the original agent of gill disease, and completely resistant to the later isolates (Marteil, 1969; Comps, 1970; Bonami, 1977). The origin of the pathogens involved has not been determined, but they may have been introduced with early imports of *C. gigas*, since that species proved to be resistant to the viral agents involved, and since early mortalities of *C. angulata* coincided with initial trial importations of *C. gigas* (Sindermann, 1976). Even though other environmental factors and industrial practices may have been involved in the decline and virtual disappearance of *C. angulata* as a species of commercial importance in France, the general opinion is that viral diseases played a significant role.

As an interesting sequel to the recent history of *C. angulata* epizootics, Comps (1980) has described an apparent decrease in the resistance of *C. gigas* to the virus. Original introductions of Pacific oysters, which reached a peak in 1970 and 1971, showed extreme resistance to the agent, but in 1977 mortalities occurred in this species too, especially in a major producing area, the Bay of Arcachon. Characteristics of the mortalities were similar to those seen in 1970 in *C. angulata*, and viral isolates had a similar morphology. *Crassostrea gigas* and *C. angulata* are closely related species, and it is not inconceivable that interbreeding of surviving *C. angulata* with the introduced species could have occurred during the intervening postepizootic years.

Other than the effects of the iridovirus on oyster (*C. angulata*) populations, probably the most interesting indication of a role for viruses in producing a severe disease in molluscs is that concerned with hemocytic neoplasms in soft-shell clams (*Mya arenaria*) (Appeldoorn and Oprandy, 1980; Oprandy *et al.*, 1981; Cooper *et al.*, 1982a,b; Oprandy and Chang, 1983). This neoplastic disease of clams occurs on the Atlantic coast of the United States and is currently at epizootic levels in some New England waters and in Chesapeake Bay. The neoplasia is progressive, leading to mortalities (Cooper *et al.*, 1982a; Farley *et al.*, 1986). According to a recent report, a B-type retrovirus has been isolated; the disease has been produced experimentally in clams injected with viruses obtained from neoplastic cells; and the virus has been reisolated from experimental clams (Oprandy *et al.*, 1981). Furthermore, neoplasia and viral activation were reported to be induced in nonneoplastic clams from endemic areas by exposure to 5-bromodeoxyuridine (a compound used to induce retrovirus expression in mammals), and neoplasia was reportedly induced in clam hemocytes *in vitro* (Oprandy and Chang, 1983). Although extensive

replications of the experiments and additional studies are needed before final assessments, this appears to be the first reported demonstration of viral induction of neoplasia in molluscs and the first transmission of neoplasia in marine invertebrates (as noted by Lauckner, 1983).

Observations of the course of epizootic hematopoietic neoplasms in soft-shell clams from New England by Cooper *et al.* (1982a) and concurrent experiments indicated three disease states: (1) progressive infections which lead to death; (2) chronic infections which may persist at a stable level for up to 10 months; and (3) remission, with diminution or disappearance of disease signs. In the study by Cooper *et al.* (1982a), 50% of diseased clams were in category 1, 40% in category 2, and 10% in category 3, with pronounced seasonal variations in severity and prevalence.

Neoplasia has been reported in six bivalve genera: *Crassostrea*, *Ostrea*, *Mytilus*, *Mya*, *Mercenaria*, and *Macoma*, as pointed out by Cooper *et al.* (1982a), but evidence for a viral etiology has been found thus far only in *Mya*, even though characteristics of the disease in other bivalves suggest an infectious etiology. Relevant too is the observation that epizootic levels of neoplasia in one species may be accompanied by absence of the disease in other bivalve species inhabiting the same area. For example, samples of *Mercenaria mercenaria* and *Macoma balthica* from Rhode Island waters, where hematopoietic neoplasia was epizootic in *M. arenaria*, were negative for the disease.

Molluscan viruses other than the iridoviruses of *C. angulata* and the presumed retrovirus of *M. arenaria* can cause pathology, but lethality has usually been demonstrated only under abnormal environmental conditions. A herpes-type virus was isolated from dying and dead oysters (*C. virginica*) being reared in heated power plant effluent in Maine by Farley *et al.* (1972). Mortalities exceeding 50% occurred in a 2-month period; histological examination disclosed intranuclear inclusions in cells around the hemolymph sinuses and massive cellular aggregates in vesicular connective tissue. Electron microscopy demonstrated viral particles with the characteristic structure of herpes-type viruses (Fig. 1). Infections were found in natural populations of oysters from the source estuary for the experimental animals (the Piscataqua River), suggesting an enzootic disease in which mortality may occur at elevated environmental temperatures. The virus has not been isolated from oysters grown elsewhere on the Atlantic coast, but Cowdry type A intranuclear inclusion bodies, similar to those seen in Maine, were reported from an oyster collected in Long Island, New York, waters (Meyers, 1981).

A lethal viral disease of oyster larvae (*C. gigas*) was described recently (Elston and Wilkinson, 1985). Labeled "oyster velar virus disease"

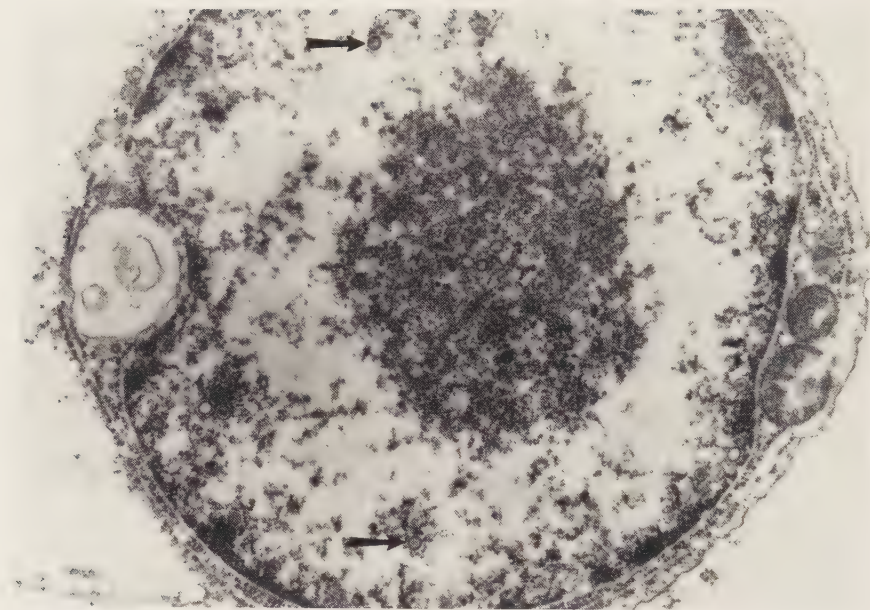


Figure 1. Herpes-like viral array in a connective tissue cell of an oyster, *Crassostrea virginica*. (Courtesy of Mr. C. A. Farley.)

and attributed to a pathogenic iridovirus infection of the larval velar epithelium, the disease was reported to have caused annual mortalities of up to 50%, during an 8-year period, in two production hatcheries on the Pacific coast of the United States. However, the disease has not been transmitted experimentally, and earlier reports of the disease (Leibovitz *et al.*, 1978; Elston, 1980) indicated a complex etiology, possibly involving a fungal agent.

A presumptive iridovirus infection of the octopus, *Octopus vulgaris*, produces gross edematous nodules on body surfaces and lesions in the muscles, beginning with the tentacles and eventually involving the mantle as well. Extensive muscle tissue necrosis, accompanied by the disgusting habit of autophagy and followed by starvation, can lead to mortalities of infected individuals. From observations in seawater tanks, time to death after the first appearance of external nodules was 3–5 months, and prevalences of 8.4% were reported in wild populations from the Bay of Naples in 1970 (Rungger *et al.*, 1971). Viral etiology was indicated by electron microscopy; membrane-bound aggregates of hexagonal viruslike particles were seen, frequently accompanied by large inclusion bodies.

Another viral infection of cephalopods—this one in cuttlefish, *Sepia officinalis*, from the French coast—was reported by Devauchelle and Vago (1971). The principal pathology was invasion and lysis of stomach cells. The presence of membrane-bound viruslike aggregates, as well as other characteristics, suggested that the agent could be a reovirus, but no isolations or transmission studies were made.

Other viruses have been seen in tissue preparations or homogenates of economically important molluscs, but have not thus far been associated consistently with pathology or mortality in natural populations. As an example, *Tellina* virus, an IPN-like agent, has been isolated from three species of oysters, a clam, and two gastropods (Buchanan, 1973; Hill, 1976a,b; Underwood *et al.*, 1977), and a reolike virus has been isolated from homogenized tissue of hatchery-reared juvenile *C. virginica* from New York (Meyers, 1979). It seems almost certain that many more viruses remain to be recognized and characterized. It is probable that some will prove to be pathogenic, or to exist in latent form, while others will prove to be only contaminants (Hill *et al.*, 1986).

Examining the list of molluscan viral diseases leads to an uneasy feeling that, while examples of pathology have been described, there is little evidence for significant levels of virus-induced mortality in natural populations, except for the *C. angulata* mortalities on the coast of France (and even here there is some question about the number of viral agents involved, as well as the possible influences of other pathogens or other environmental conditions). Certainly, more experimental transmission studies are needed, as are other approaches to elucidating the epidemiology of each proposed agent.

Recent reports of a lethal viral disease of larvae in molluscan hatcheries do indicate a little of what may be expected in the future and do suggest a course of prophylactic action. Elston and Wilkinson (1985) have outlined steps in the management and eradication of a viral disease in culture facilities. They are:

- Assuming the pathogen is highly infectious and lethal to larvae, the best management strategy is destruction of infected larval populations and sterilization of equipment.
- Eradication of the disease from brood stock is a concurrent high-priority requirement. Possible actions to bring this about include:
 1. identification of suspect brood stock, assuming that a carrier state may exist
 2. isolation of suspect brood stock and offspring to control spread of the pathogen
 3. diagnosis of larval disease

4. destruction of infected brood stock and larvae and sterilization of all equipment
5. identification and maintenance of a disease-free brood stock

The authors pointed out that these measures may not be economically feasible in production hatcheries, and that continuing research is needed on matters such as the diagnosis of carriers, methods of eliminating the disease from carriers, mechanisms of disease resistance, and identification of possible alternate hosts.

III. VIRAL DISEASES OF CRUSTACEANS

The first viral disease reported from crustaceans—in fact, the first viral disease reported in any marine invertebrate—was found by Vago (1966) in the shore crab, *Macropipus (Portunus) depurator*, from the French coast. During the subsequent two decades, knowledge of crustacean viruses has expanded remarkably, so that now more than 30 have been described in varying detail from wild and cultured hosts, and undoubtedly many more will be recognized. The status of knowledge about crustacean viruses has been reviewed by Couch (1981), Johnson (1983, 1984), and Sparks (1985). The families of viruses represented in marine invertebrates are listed in Table 2, and information about those from Crustacea has been summarized in Table 3.

It should be noted at the outset, though, that, as with the molluscan viruses, most descriptions of crustacean viruses are based on information from electron microscopy; viral isolations and transmission studies with such isolates have yet to be accomplished. Furthermore, no crustacean cell culture systems are currently available for viral studies, and

Table 2. Viral Families Represented in Marine Invertebrates

DNA Viruses	RNA Viruses
Herpesviridae (N, C)	Reoviridae (C)
Baculoviridae (N, C)	Birnaviridae (C)
	Bunyaviridae (C)
	Rhabdoviridae (C)
	Picornaviridae (C)

N, Nuclear; C, cytoplasmic.

Table 3. Viruses from Marine Crustacea

Host	Virus Type	Location in Host and Pathology	Effect on Host	Geographic Distribution	References
<i>Penaeus duorarum</i> (pink shrimp) (also occurs in <i>P. setiferus</i> , <i>P. aztecus</i> , <i>P. vannamei</i> , and <i>P. stylirostris</i>)	<i>Baculovirus penaei</i> (BP) (occluded)	Nuclei of hepatopancreatic cells; tetrahedral inclusion bodies prevalent	Larvae, juveniles, and adults are susceptible; no gross lesions; infected nuclei enlarged, with degeneration of nucleoli; infections in natural populations may reach 80%, with subsequent mortalities	Gulf of Mexico	Couch (1974a,b, 1978, 1981), Couch and Courtney (1977), Couch <i>et al.</i> (1975), Lightner <i>et al.</i> (1983b)
<i>Penaeus monodon</i> (also seen in <i>P. merguensis</i>)	Baculovirus (occluded), called monodon baculovirus (MBV)	Hepatopancreas and mid-gut epithelium; spherical inclusion bodies formed; intranuclear	Necrosis of hepatopancreatic tubules, followed by hemocytic encapsulation; heavily infected individuals lethargic; mortalities occur in high-density culture	Pacific coast of Mexico, Hawaii; wild populations and culture facilities in Taiwan, Philippines, Tahiti	Lightner and Redman (1981), Lightner <i>et al.</i> (1983a,b)
<i>Penaeus japonicus</i> (Kuruma prawn)	Baculovirus (nonoccluded), called baculoviral mid-gut necrosis virus (BMNV) Reo-like, called REO	Nuclei of hepatopancreas, resulting in hypertrophy, but no inclusion bodies are formed Found in hepatopancreatic cells, produces cytoplasmic inclusion bodies; results in necrosis and	Causes severe mortalities in cultured larvae and postlarvae, resulting from hepatopancreatic necrosis REO found in association with "gut and nerve" syndrome in postlarvae and juveniles;	Japan Hawaii, France; may be enzootic in Japan	Sano <i>et al.</i> (1981) Lightner (1988a,b), Lightner <i>et al.</i> (1984), Tsing <i>et al.</i> (1985), Tsing

(continued)

Table 3. (Continued)

Host	Virus Type	Location in Host and Pathology	Effect on Host	Geographic Distribution	References
<i>P. stylirostris</i> (Pacific blue shrimp) (also occurs in <i>P. monodon</i> and <i>P. vannamei</i>)	Parvo or picorna-virus groups, called infectious hypodermal and hematopoietic necrosis virus (IHHNV)	atrophy of the hepatopancreas Nuclei of ectodermally and mesodermally derived tissues; large inclusion bodies of Cowdry type A formed	causes lethargy, abnormal behavior, and death Causes diffuse tissue necrosis with generalized inflammation; severe acute disease of juvenile <i>P. stylirostris</i> ; transmitted vertically	West coast of Central and South America; also introduced into culture facilities in Hawaii	Lightner <i>et al.</i> (1983b,c), Bell and Lightner (1983, 1984), Lightner <i>et al.</i> (1985)
<i>Penaeus merguensis</i> , <i>P. semisulcatus</i> , <i>P. orientalis</i> , <i>P. monodon</i>	Parvovirus, called hepatopancreatic parvo-like virus (HPV)	Hepatopancreatic tubule epithelial cells, with prominent intranuclear inclusion bodies	Causes mortalities of juveniles, especially in <i>P. merguensis</i> and <i>P. semisulcatus</i> ; mortalities may reach 100% in 1-2 months	Singapore, Kuwait, China, Philippines	Lightner and Redman (1985)
<i>Penaeus aztecus</i> (brown shrimp)	Size range of picorna- and parvoviruses	Phagosomes of fixed phagocytes of heart	Not reported; could be phages within partially lysed bacteria	Gulf of Mexico	Foster <i>et al.</i> (1981)
<i>Callinectes sapidus</i> (blue crab)	Reo-like, hence called RLV (non-enveloped, icosahedral), similar to paralysis virus of <i>Macropipus depurator</i> in France	Principal sites of infection are hemocytes and hematopoietic tissue, but also found in several other tissues. Always found in association with a second virus—rhabdo-like virus A (RhVA) and occasionally with other viruses	Causes necrosis of hematopoietic tissue and hemocytes; causes paralysis and death, probably as consequence of nerve cell destruction	Chincoteague Bay, Virginia; Chesapeake Bay	Johnson (1977a,b), Johnson and Bodammer (1975)

Picorna-like virus, called Chesapeake Bay virus (CBV)	Infects cells of ectodermal origin—epidermis, gill epithelium, nerve cells, gut epithelium	Extensive destruction of gill epithelium and neurosecretory cells; may produce blindness and death. Often found in conjunction with other viruses	Chesapeake Bay	Johnson (1978a,b)
Enveloped helical virus, with similarities to Paramyxoviridae (EHV)	Infects hemocytes and hematopoietic tissue	Pathology unreported; always found in association with other viruses (which may cause the pathology seen)	Chesapeake Bay	Johnson and Farley (1980)
Rhabdo-like virus A (RhVA)	Found in Schwann cells, hemocytes, fibroblasts, blood vessel endothelia, and other cell types	Pathology unreported, but may act with other crab viruses in producing disease	Atlantic coast of U.S.; Gulf of Mexico	Jahromi (1977), Yudin and Clark (1978, 1979), Johnson (1976a-c, 1983)
Baculovirus A (non-occluded)	Nuclei of hepatopancreatic epithelial cells	Causes nuclear hypertrophy, affects adults and juveniles; infection prevalences up to 52%, but no observable effect on hosts	Atlantic coast of U.S.	Johnson (1976a-c)
Baculovirus B (non-occluded), similar to baculovirus of <i>Carcinus maenas</i>)	Found in hemocytes and hematopoietic tissues	Causes nuclear hypertrophy and lysis of hemocytes, but effects on host otherwise unknown	Chesapeake Bay	Johnson (1983), Bazzin <i>et al.</i> (1974)

(continued)

Table 3. (Continued)

Host	Virus Type	Location in Host and Pathology	Effect on Host	Geographic Distribution	References
<i>Macropipus depurator</i>	Bifaciens virus (BFV) possibly representing an undescribed family	Infects nuclei of hemocytes and occasionally other cells; large refractile inclusions in cytoplasm	Causes nuclear hypertrophy followed by lysis of cell; infected crabs have milky hemolymph; mortalities may occur; infections up to 13% reported	Chincoteague Bay, Virginia; Assawoman Bay, Delaware	Johnson (1976a, 1983, 1988)
	Rhabdo-like virus, labeled EGV-1 and later RhVB)	Associated with basal lamina of mandibular glands	Not reported	Atlantic coast of U.S.	Yudin and Clark (1978), Johnson (1983)
	Reoviridae, called paralysis virus, or P virus	Connective tissue and hemocytes	Causes paralysis and disruption of gill tissues; heavy infections occur in interstitial tissues of hepatopancreas; frequently associated with S-virus; may cause mortalities	French Mediterranean coast	Vago (1966), Bonami (1973), Bonami <i>et al.</i> (1976)
So-called V31-V24 complex viruses—not further classified	S-virus, with characteristics of Bunyaviridae and Paramyxoviridae	Cardiac tissue, endothelial cells of hepatopancreatic blood vessels, and hemocytes	Infected crabs weaken and die, with no obvious signs of disease; experimental infections produce mortalities	French Mediterranean coast	Bonami and Vago (1971), Bonami <i>et al.</i> (1971, 1975)
		Not reported	Experimental inoculation of material from diseased crabs reproduced the disease	France	Bonami (1976)

<i>Carcinus maenas</i> (European shore crab)	Virus with characteristics of Bunyaviridae, called crab hemocytopenic virus (CHV)	Hemocytes	Inhibits clotting of hemolymph, reduces numbers of circulating hemocytes, and may cause death	Brittany coast of France	Bang (1971, 1974), Hoover (1977), Hoover and Bang (1976, 1978)
	Baculovirus (nonoccluded)	Nuclei of hemocytes and connective tissue cells in areas of regeneration following autotomy	Nuclear hypertrophy; other effects not reported	France	Bazin <i>et al.</i> (1974)
	Rhabdoviridae? (presumptive), called Y-organ virus; similar to the S-virus of <i>M. depurator</i>	Beneath basal lamina of Y-organ	Not reported	Brittany coast of France	Chassard-Bouchaud and Hubert (1975), Chassard-Bouchaud <i>et al.</i> (1976)
<i>Carcinus mediterraneus</i>	Reoviridae (presumptive), called gill virus	Gill epithelial cells	Infection causes lysis of gill epithelial cells; experimental infections cause death in 8 days	French Mediterranean coast	Bonami (1976)
	Baculovirus (tau)	Nuclei of hepatopancreatic and midgut epithelial cells	Causes nuclear hypertrophy, cell lysis, and mortality after experimental transmission	French Mediterranean coast	Pappalardo and Bonami (1979), Mari and Bonami (1986)
	Y-organ virus (cytoplasmic) morphologically similar to Y-organ virus of <i>C. maenas</i>	Y-organ; develops in cytoplasmic vacuoles of glandular cells	Not reported	French Mediterranean coast	Zerbib <i>et al.</i> (1975), Bonami (1976)

(continued)

Table 3. (Continued)

Host	Virus Type	Location in Host and Pathology	Effect on Host	Geographic Distribution	References
<i>Paralithodes platypus</i> (blue king crab) (also seen in <i>P. camtschatica</i> and <i>Lithodes aequispina</i>)	Herpeslike (presumptive)	Epithelium of bladder, antennal gland and hindgut, with nuclear hypertrophy and ovoid inclusion bodies	Causes destruction of bladder and antennal gland; may be implicated in population declines	Alaska	Sparks (1985), Sparks and Morado (1985, 1986)
<i>Rhithropanopeus harrisi</i>	Herpeslike virus	Mesodermal cells of male gonad	Degeneration of infected cells	North Carolina	Payen and Bonami (1979)



Figure 2. Polyhedral inclusion bodies associated with *Baculovirus penaei* infection of a shrimp hepatopancreatic cell. (Courtesy of Dr. D. V. Lightner.)

even primary explant methods are inadequate. The techniques used at present for recognition and diagnosis of crustacean viruses include:

1. Observing gross signs and behavioral changes which have been found to be characteristic of some viral infections
2. Observing the rates and patterns of mortalities in the several life history stages of suspect populations, which may be characteristic of particular viral diseases
3. Light microscopy, useful in detecting the presence, location, and form of inclusion bodies that are characteristic of some infections (Fig. 2) and in recognizing other distinctive pathological changes in host cells and tissues
4. Electron microscopy, to examine ultrastructural characteristics of virions and intracellular changes resulting from infections (Fig. 3)
5. Enhancement studies, stressing the animals by overcrowding, abnormal temperatures, or sublethal doses of toxic chemicals to increase the prevalence and severity of infections

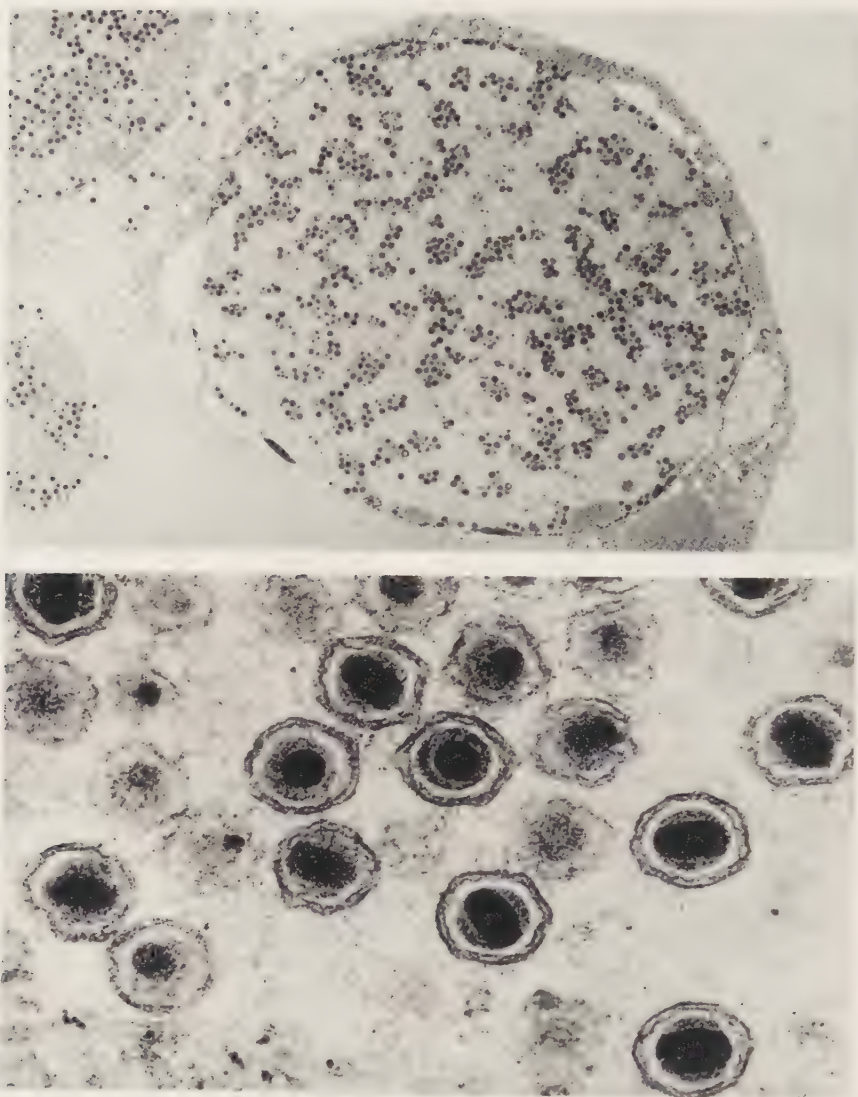


Figure 3. Electron micrographs of nucleus of a blue crab hemocyte, hypertrophied and containing bifacies virus particles (above), with enlargement of the virions (below). (Courtesy of Dr. P. T. Johnson.)

Table 4. Status of Knowledge about Crustacean Viruses

State of Knowledge	Disease Agent
Limited to observation of presumed viral particles	Y-organ viruses of <i>Carcinus maenas</i> and <i>Carcinus mediterraneus</i> , baculovirus of <i>C. maenas</i> , herpes-like virus of <i>Paralithodes platypus</i>
Infectious agent purified by ultrafiltration and used for experimental demonstration of transmission of disease	V31–V24 complex viruses of <i>Macropipus depurator</i>
Preliminary ultrastructural and biochemical characterization	Baculovirus of <i>C. maenas</i> , gill virus of <i>C. mediterraneus</i> , reo-like virus of <i>Callinectes sapidus</i> , bifacies virus of <i>C. sapidus</i> , baculovirus of <i>C. sapidus</i> , infectious hypodermal and hematopoietic necrosis virus of <i>Penaeus stylirostris</i> , hepatopancreatic parvo-like virus of <i>Penaeus merguensis</i>
Characterization still incomplete but adequate to consider taxonomic position, based on electron microscopy, biochemical studies, and experimental transmission studies	<i>Baculovirus penaei</i> of <i>Penaeus duorarum</i> , paralysis virus of <i>M. depurator</i> , S-virus of <i>M. depurator</i> , baculovirus of <i>Penaeus monodon</i> , baculovirus of <i>Penaeus japonicus</i>

6. Carrier detection bioassays, exposing larvae or juveniles of other species to adult individuals suspected of harboring viral infections in latent form. Exposures can be *oral*, by feeding macerated suspect individuals; by *direct contact*, by adding suspect individuals to a susceptible population; or by *indirect contact*, by adding effluent water from tanks holding suspect animals to another tank containing the susceptible population.

The relative states of knowledge about viruses of crustaceans have been categorized in Table 4.

Most of the viruses or presumed viruses of marine Crustacea have been recognized histopathologically by the presence of inclusion bodies, or by electron microscopy. Since crustacean tissue cultures are unavailable, characterization has not been feasible beyond the information derived from microscopy and from transmission experiments with ultrafiltrates of infected tissues. Despite these severe limitations, interest in crustacean virology is expanding, especially that concerned with the pathology of cultured species such as shrimp.

So-called "enhancement" studies, in which latent infections are

provoked into patency by abnormal environmental conditions, have yielded some information about viral diseases. For example, prevalences and effects of the pink shrimp (*Penaeus duorarum*) baculovirus can be increased by exposure to sublethal doses of toxic chemicals (polychlorinated biphenyls and Mirex) or overcrowding (Couch, 1974a,b; Couch and Nimmo, 1973). Most of the very productive studies of blue crab viruses by Johnson (1978a, 1983) were conducted on captive populations confined in laboratory tanks under stress-inducing conditions of overcrowding. In fact, it seems that most of the pathogenic viruses described thus far from Crustacea have been associated with some form of stress—crowding, nonoptimum holding or culture conditions, exposure to toxic chemicals, concurrent infections, limb autonomy, or eyestalk ablation. Thus, in addition to the excellent example of increased prevalences of patent infections of pink shrimp by *Baculovirus penaei*, seen after crowding or exposure to chemical stressors (Couch, 1974b), the literature contains reports of proliferation of a rhabdo-like virus in the mandibular glands of blue crabs, *Callinectes sapidus*, after eyestalk removal (Yudin and Clark, 1979), and a baculovirus of shore crabs, *Carcinus maenas*, discovered in regenerating tissue following experimentally induced autonomy of appendages (Bazin *et al.*, 1974). This does not imply, however, that *all* viral diseases of Crustacea are stress related, since factors of innate resistance may be important as well.

The first virus of Crustacea, as described by Vago (1966) in the European shore crab, produced gross signs of disability and resulted in mortality. Gross signs included the slow development of paralysis, and sometimes a slight darkening of the exoskeleton in later phases of the disease. Viral particles were seen with the electron microscope; inoculation of blood from infected animals produced disease signs in healthy crabs; and infections were obtained with ultrafiltrates and ultracentrifugates of homogenized tissue from sick crabs. No indication of disease prevalence was given by Vago. In the same year, Gordon (1966) made fleeting reference to a nonpathogenic virus in shrimp from the east coast of Africa. The report, without substantiating evidence, merely stated that the virus occurred frequently in *Penaeus indicus* in the Rufiji Delta, its presence being indicated by a "whitish blotchy disfigurement dorsally under the integument." Further study might disclose other causes for the condition (such as microsporidiosis); the report is mentioned here only because of the extreme paucity of historical information about viruses in marine invertebrates.

Since the original description of the highly pathogenic viral disease of crabs by Vago, other mortality-associated viruses have been recognized. Three viruses have been reported to cause fatal infections in blue

crabs, *C. sapidus*, from the east coast of the United States, in the studies by Johnson and associates (1973–1980), while two other viruses have been demonstrated to kill the Mediterranean shore crab, *Carcinus mediterraneus* (Bonami, 1976; Pappalardo and Bonami, 1979).

It is important to note, though, that not all of the viruses of crabs described to date have been demonstrated to be pathogenic or to cause fatal infections. As Johnson (1978a) pointed out, effects on the host may be unknown, may be relatively benign, may result from synergism with other viruses, or may be lethal. As an example, of the seven viruses of the blue crab, *C. sapidus*, she studied, two were relatively benign (baculovirus A and B), two were found to be associated with other viruses (paramyxo-like virus and rhabdo-like virus), while three others were lethal after causing progressive disability (reo-like virus, bifacies virus, and picorna-like virus). The rhabdo-like virus apparently acted synergistically with the reo-like virus and may have been responsible for neural components of the disease syndrome which resulted from concurrent infections. Johnson concluded that multiple infections were the rule in her material. Of 21 laboratory-held virally infected crabs examined by electron microscopy, three different viruses were found in 12 specimens; two different viruses existed in seven; and only two crabs had just a single virus. Postcapture transmission may have occurred in at least some instances.

Among penaeid shrimp, attention has naturally focused on viruses which may cause mortality, because of the economic importance of the host species and the great potential of shrimp in aquaculture production (Lightner, 1983). Thus far, six fatal viral diseases of penaeids have been reported (Table 3). Larvae and juveniles are most frequently infected, as might be expected, and it appears that individual and species variations in resistance can occur, resulting in carrier states which may have serious implications for the spread of the agents involved.

From extensive studies of shrimp viruses, a number of conclusions may be drawn³—conclusions which may prove applicable to other crustacean viruses as well:

- Viral diseases and associated mortalities are emerging as one of the most important problems in penaeid shrimp culture.
- Each virus is infective for at least one, but usually several, host species, which may exhibit varying degrees of resistance.
- A viral agent may be harbored as an inapparent infection during

³This list of tentative conclusions has been extracted, modified, and expanded from an excellent overview paper by Brock (1983).

parts of the host's life cycle, but may cause patent disease and resultant mortalities at particular life cycle stages.

- Some host species may serve as inapparent carriers, which rarely develop patent disease from the viral infection, but which may transmit the infection to more susceptible species.
- Viral disease outbreaks may be directly related to the degree of stress on the host populations. Common stressors in culture environments include high population density, abnormal temperatures, and hypoxia.
- Viral detection by currently available methods may be possible only if hosts are examined during the periods of susceptibility to acute disease.
- Cell culture and immunological procedures—critical to vertebrate virology—are currently unavailable for marine invertebrate virology. Diagnosis now depends on gross pathology, histopathology (particularly differential staining and demonstration of inclusion bodies), electron microscopy, and (when feasible) transmission studies using ultrafiltrates of presumed infective material.
- Whenever animals are transported from one area to another, as is often done for aquaculture purposes, the risk exists for introducing unknown or inapparent viral diseases, which may have severe effects on susceptible species in the recipient area. Countermeasures include whole-life-cycle quarantine and the use of presumably disease-free source stocks, recognizing the limitations of current knowledge about viral pathogens of Crustacea.

Other generalizations, with varying amounts of factual foundation, are:

- Many of the viruses which have been seen in Crustacea occur concurrently with other viruses, some with great consistency (Fig. 4). Such associations suggest possible synergisms, or that particular associations may be necessary for viral expression (Johnson, 1983; Mari and Bonami, 1986).
- Baculovirus infections have proved to be common in Crustacea, as they are in other arthropods. Based on present information, the baculoviruses seem to be the most important of all the viral groups that are found in Crustacea—in both numbers and effects.
- Frequent sites of viral infections in Crustacea are hemocytes and hepatopancreatic cells.

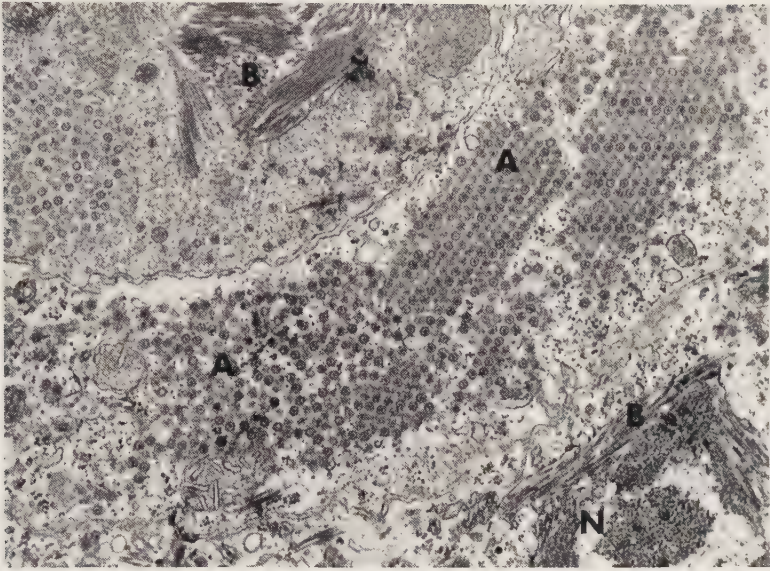


Figure 4. Arrays of reo-like virus (A) and rhabdo-like virus (B) in a hemopoietic cell of a blue crab. (Courtesy of Dr. P. T. Johnson.)

- The association of some viral infections with mortalities can be most readily demonstrated in captive populations, but viruses may have been implicated in many previously unexplained mortalities in feral and cultured populations.
- The distribution of recognized viruses of crabs and shrimps parallels the distribution and range of scientists with special interests in those groups. So, for example, knowledge of viruses of crabs in Chesapeake Bay and adjacent waters is principally a consequence of the work of P. T. Johnson and associates; knowledge of the viruses of crabs on the French Mediterranean coast can be attributed to the work of C. Vago, J. R. Bonami, and associates; while knowledge of viruses in North American penaeid shrimps has been developed by J. A. Couch, D. V. Lightner, and their associates. This is not an uncommon phenomenon in other developing specialty areas in science, in which frequencies of observations are proportional to geographic distributions of observers (and, in the case of marine animals, the economic significance of the host species).

IV. CONCLUSIONS

Viruses have been reported with increasing frequency in molluscan and crustacean shellfish during the past two decades. Some have been associated, presumptively, with disease, although experimental infections have been attempted only rarely. Usually, diagnosis is based on histopathology, electron microscopy, and some limited biochemistry. Presumed viral agents have not been isolated, since stable cell culture systems for shellfish do not exist and antigenic characterization is not yet practicable.

Of those diseases of molluscs which seem to have an association with viruses, epizootics of an irido-like virus in Portuguese oysters, *Crassostrea angulata*, have provided the most dramatic example of widespread major mortalities. Cultivation of that species on the coast of France was virtually eliminated by successive outbreaks of iridoviruses which caused gill disease in the late 1960s and early 1970s.

Among viral diseases of crustaceans, *Baculovirus penaei*, which may cause mortalities in Gulf of Mexico pink shrimp, is probably the best known, although an array of other viruses has been reported for shrimp and crabs. Most of the viral infections have been recognized in culture systems for shrimp or in impounded populations of crabs.

Indications exist that viral diseases may become important in intensive culture of oysters and shrimp. An iridovirus was identified recently as the cause of larval mortalities in Pacific oyster (*Crassostrea gigas*) hatcheries, and six fatal viral diseases of penaeid shrimp have been recognized during the past decade (Lightner, 1983; Bonami, 1987). Dominant viral groups appear to be the iridoviruses in molluscs and the baculoviruses in crustaceans.

Viruses may be present in shellfish as virulent pathogens, as latent infections, or as harmless contaminants. Detection of carriers of potential pathogens can be a critical research activity, especially in the selection of hatchery brood stock. Recognition of those viruses which may be present in the animal simply as contaminants (birnaviruses in molluscs, for example) can also be important to understanding possible disease transmission in other marine populations. It seems probable that many other viruses will be discovered in shellfish; it is also probable that some of the mass mortalities of earlier years may have resulted from outbreaks of viral diseases.

REFERENCES

- Alderman, D. J. (1980). Shellfish disease problems—Past, present and future. *Proc. Annu. Conf. Shellfish. Assoc. Great Britain*, 11th pp. 30–40.

- Alderman, D. J., and Gras, P. (1969). Gill disease of Portuguese oysters. *Nature (London)* **224**, 616–617.
- Appeldoorn, R. S., and Oprandy, J. J. (1980). Tumors in soft-shell clams and the role played by a virus. *Maritimes* **24**, 4–6.
- Appeldoorn, R. S., Brown, R. S., and Brown, C. W. (1979). A three-month growth and mortality study of normal and neoplastic *Mya arenaria* cross-transplanted between clean and oil-impacted areas. *Proc. Natl. Shellfish. Assoc.* **69**, 192.
- Arvy, L., and Franc, A. (1968). Sur un protiste nouveau, agent de destruction des branchies et des palpes de l'huître portugaise. *C. R. Hebd. Seances Acad. Sci., Ser. D* **267**, 103–105.
- Bang, F. B. (1971). Transmissible disease, probably viral in origin, affecting the amoebocytes of the European shore crab, *Carcinus maenas*. *Infect. Immun.* **3**, 617–623.
- Bang, F. B. (1974). Pathogenesis and autointerference in a virus disease of crabs. *Infect. Immun.* **9**, 1057–1061.
- Barry, M. M., and Yevich, P. P. (1972). Incidence of gonadal cancer in the quahaug *Mercenaria mercenaria*. *Oncology* **26**, 87–96.
- Bazin, F., Monsarrat, P., Bonami, J. R., Croizier, G., Meynadier, G., Quiot, G., and Vago, C. (1974). Particules virales de type baculovirus observées chez le crabe *Carcinus maenas*. *Rev. Trav. Inst. Pêches Marit.* **38**, 205–208.
- Bell, T. A., and Lightner, D. V. (1983). The penaeid shrimp species affected and known geographic distribution of IHHN virus. *Proc. Conf. Warm Water Fish Culture—Crustaceans*, 1st 12 pp.
- Bell, T. A., and Lightner, D. V. (1984). IHHN virus: Infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. *Aquaculture* **38**, 185–194.
- Besse, P. (1968). Résultats de quelques observations sur une affection branchiale des huîtres (*Crassostrea angulata* Lmk.). *Bull. Acad. Vet. Fr.* **41**, 87–91.
- Bonami, J.-R. (1973). Recherche sur la paralysie virale du crustacé décapode *Macropipus depurator* L. *Rev. Trav. Inst. Pêches Marit.* **37**, 387–389.
- Bonami, J.-R. (1976). Viruses from crustaceans and annelids: Our state of knowledge. *Proc. Int. Colloq. Invertebr. Pathol., 1st—Annu. Meet. Soc. Invertebr. Pathol.*, 9th pp. 20–23.
- Bonami, J.-R. (1977). Les maladies virales des crustacés et des mollusques. *Oceanis* **3**, 154–175.
- Bonami, J.-R. (1987). Les affections virales des crevettes penaeides. *Oceanis* **13**, 233–245.
- Bonami, J.-R., and Vago, C. (1971). A virus of a new type pathogenic to Crustacea. *Experientia* **27**, 1363–1364.
- Bonami, J.-R., Vago, C. and Duthoit, J. L. (1971). Une maladie virale chez les crustacés décapode due a un virus d'un type nouveau. *C. R. Hebd. Seances Acad. Sci., Ser. D* **272**, 3087–3088.
- Bonami, J.-R., Veyrunes, J. C., Cousserans, F., and Vago, C. (1975). Ultrastructure, developpement et acide nucléique du virus S du crustacé décapode *Macropipus depurator* L. (Crustacé, Décapode). *C. R. Hebd. Seances Acad. Sci., Ser. D* **280**, 359–361.
- Bonami, J.-R., Comps, M., and Veyrunes, J. C. (1976). Étude histopathologique et ultrastructure de la paralysie virale du crabe *Macropipus depurator* L. *Rev. Trav. Inst. Pêches Marit.* **40**, 139–146.
- Brock, J. A. (1983). An overview of penaeid viral diseases and comments regarding prevention of the introduction of these viruses with imports of live penaeid shrimp. *Workshop Doc., Pacific Mar. Fish. Comm., Subcomm. Shellfish Transfer* 4 pp.
- Buchanan, J. S. (1973). Electron microscopic observations of virus-like particles in the digestive gland of a marine bivalve, *Tellina tenuis*. *Proc. Int. Colloq. Insect Pathol. Microb. Control*, 5th p. 28.

- Buchanan, J. S., and Richards, R. H. (1982). Herpes-type virus diseases of marine organisms. *Proc.—R. Soc. Edinburgh, Sect. B* **81**, 151–168.
- Chassard-Bouchaud, C., and Hubert, M. (1975). Sur l'existence de vésicules de réticulum endoplasmique lisse dans l'organe Y de *Carcinus maenas* L. (Crustacé Décapode). *C. R. Hebd. Seances Acad. Sci., Ser. D* **281**, 707–709.
- Chassard-Bouchaud, C., Hubert, M., and Bonami, J. R. (1976). Particules d'allure virale associées à l'organe Y du crabe *Carcinus maenas* (Crustacé, Décapode). *C. R. Hebd. Seances Acad. Sci., Ser. D* **282**, 1565–1566.
- Comps, M. (1969). Observations relatives à l'affection branchiale des huîtres portugaises (*Crassostrea angulata* Lmk.). *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **33**, 151–160.
- Comps, M. (1970). La maladie des branchies chez les huîtres du genre *Crassostrea*. Caractéristiques et évolution des altérations, processus de cicatrisation. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **34**, 23–43.
- Comps, M. (1972). Observations sur la résistance d'huîtres du genre *Crassostrea* au cours de la mortalité massive de 1970–1971 dans le bassin de Marennes-Oléron. *Int. Counc. Explor. Sea, Doc. C.M.1972/K*, 22.
- Comps, M. (1978). Évolution des recherches et études récentes en pathologie des huîtres. *Oceanol. Acta* **1**, 255–262.
- Comps, M. (1980). Les infections virales associées aux épizooties des huîtres du genre *Crassostrea*. *Int. Counc. Explor. Sea, Spec. Meet. Dis. Commer. Important Mar. Fish Shellfish* **6**.
- Comps, M. (1983). Less infections virales associées aux épizooties des huîtres du genre *Crassostrea*. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **182**, 137–139.
- Comps, M., and Bonami, J. R. (1977). Infection virale associée à des mortalités chez l'huître *Crassostrea gigas* Thunberg. *C. R. Hebd. Seances Acad. Sci., Ser. D* **185**, 1139–1140.
- Comps, M., and Duthoit, J. L. (1976). Infection virale associée à la maladie des branchies de l'huître portugaise *Crassostrea angulata* Lmk. *C. R. Hebd. Seances Acad. Sci., Ser. D* **283**, 1595–1596.
- Comps, M., and Duthoit, J. L. (1979). Infections virales chez les huîtres *Crassostrea angulata* Lmk. et *Crassostrea gigas* Th. *Haliotis* **8**, 301–307.
- Comps, M., Bonami, J.-R., and Campillo, A. (1976). Une virose de l'huître portugaise (*Crassostrea angulata* Lmk.). *C. R. Hebd. Seances Acad. Sci., Ser. D* **282**, 1991–1993.
- Cooper, K. R., Brown, R. S., and Chang, P. W. (1982a). The course and mortality of a hematopoietic neoplasm in the soft-shell clam, *Mya arenaria*. *J. Invertebr. Pathol.* **39**, 149–157.
- Cooper, K. R., Brown, R. S., and Chang, P. W. (1982b). Accuracy of blood cytological screening techniques for the diagnosis of a possible hematopoietic neoplasm in the bivalve mollusc, *Mya arenaria*. *J. Invertebr. Pathol.* **39**, 281–289.
- Couch, J. A. (1974a). Free and occluded virus, similar to *Baculovirus*, in hepatopancreas of pink shrimp. *Nature (London)* **247**, 229–231.
- Couch, J. A. (1974b). An enzootic nuclear polyhedrosis virus of pink shrimp: Ultrastructure, prevalence, and enhancement. *J. Invertebr. Pathol.* **24**, 311–331.
- Couch, J. A. (1978). Diseases, parasites and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic Coasts of North America. *Fish. Bull.* **76**, 1–44.
- Couch, J. A. (1981). Viral diseases of invertebrates other than insects. In "Pathogenesis of Invertebrate Microbial Diseases" (E. W. Davidson, ed.), pp. 127–160. Allanheld, Osmun Publ., Totowa, New Jersey.
- Couch, J. A., and Courtney, L. (1977). Interaction of chemical pollutants and virus in a crustacean: A novel bioassay system. *Ann. N.Y. Acad. Sci.* **298**, 497–504.
- Couch, J. A., and Nimmo, D. R. (1973). Cytopathology, ultrastructure and virus infection in pink shrimp exposed to the PCB, Aroclor 1254. *Proc. Int. Colloq. Insect Pathol.* p. 105 (Abstr.).

- Couch, J. A., Summers, M. D. and Courtney, L. (1975). Environmental significance of *Baculovirus* infections in estuarine and marine shrimp. *Ann. N.Y. Acad. Sci.* **260**, 528–536.
- Deltreil, J. P. (1969). Remarques sur la croissance en élevage suspendu de *Crassostrea angulata* Lmk. affectée par la maladie des branchies dans le bassin d'Arcachon. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **33**, 176–180.
- Deltreil, J. P. (1971). Etude comparée du comportement de trois lots d'huîtres portugaises *Crassostrea angulata* Lmk. de différentes origines en relation avec l'évolution de la maladie des branchies dans le bassin d'Arcachon. Essai de bilan et conséquences. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **35**, 89–97.
- Devauchelle, G., and Vago, C. (1971). Particules d'allure virale dans les cellules de l'estomac de la seiche, *Sepia officinalis* L. (mollusques, céphalopodes). *C. R. Hebd. Seances Acad. Sci., Ser. D* **272**, 894–896.
- Elston, R. A. (1979). Viruslike particles associated with lesions in larval Pacific oysters (*Crassostrea gigas*). *J. Invertebr. Pathol.* **33**, 71–74.
- Elston, R. A. (1980). Ultrastructural aspects of a serious disease of hatchery reared larval Pacific oysters, *Crassostrea gigas*. *Proc. Natl. Shellfish. Assoc.* **70**, 122.
- Elston, R. A., and Wilkinson, M. T. (1985). Pathology, management and diagnosis of oyster velar virus disease (OVVD). *Aquaculture* **48**, 189–210.
- Farley, C. A. (1973). Virus diseases of oysters. *Wildl. Dis. Conf.* p. 19.
- Farley, C. A. (1975). Electron microscopy of virus infections in oysters (*Crassostrea virginica*). *Proc. Soc. Invertebr. Pathol. Annu. Meet.* pp. 28–29. (Abstr.).
- Farley, C. A. (1976a). Ultrastructural observations on epizootic neoplasia and lytic virus infection in bivalve mollusks. *Prog. Exp. Tumor Res.* **20**, 283–294.
- Farley, C. A. (1976b). Proliferative disorders in bivalve mollusks. *Mar. Fish. Rev.* **38**, 30–33.
- Farley, C. A. (1977). Neoplasms in estuarine mollusks and approaches to ascertain causes. *Ann. N.Y. Acad. Sci.* **298**, 225–232.
- Farley, C. A. (1978). Viruses and viruslike lesions in marine mollusks. *Mar. Fish. Rev.* **40**, 18–20.
- Farley, C. A. (1981). Phylogenetic relationships between viruses, marine invertebrates and neoplasia. In "Phyletic Approaches to Cancer" (C. J. Dawe, J. C. Harshbarger, S. Kondo, T. Sugimura, and S. Takayama, eds.), pp. 75–87.
- Farley, C. A., Banfield, W. G., Kasnic, G., Jr., and Foster, W. S. (1972). Oyster herpes-type virus. *Science* **178**, 759–760.
- Farley, C. A., Otto, S. V., and Reinisch, C. L. (1986). New occurrence of epizootic sarcoma in Chesapeake Bay soft shell clams, *Mya arenaria*. *Fish. Bull.* **84**, 851–857.
- Foster, C. A., Farley, C. A., and Johnson, P. T. (1981). Virus-like particles in cardiac cells of the brown shrimp, *Penaeus aztecus* Ives. *J. Submicrosc. Cytol.* **13**, 723–726.
- Franc, A., and Arvy, L. (1969). Sur *Thanatostrea polymorpha* n. g., n. sp., agent de destruction des branchies et des palpes de l'huître portugaise. *C. R. Hebd. Seances Acad. Sci., Ser. D* **268**, 3189–3190.
- Franc, A., and Arvy, L. (1970). Données sur l'évolution de la maladie des branchies chez les huîtres et sur son agent causal: *Thanatostrea polymorpha* Franc et Arvy, 1969. *Bull. Biol. Fr. Belg.* **104**, 2–19.
- Gordon, I. (1966). Parasites and diseases of Crustacea. *Mem. Inst. Fond. Afr. Noire* **77**, 27–86.
- Gras, P. (1969). Recherches sur l'organisme responsable de la maladie des branchies. *Rev. Trav. Int. Sci. Tech. Pêches Marit.* **33**, 161–164.
- Harshbarger, J. C., Otto, S. V., and Chang, S. C. (1979). Proliferative disorders in *Crassostrea virginica* and *Mya arenaria* from the Chesapeake Bay and intranuclear virus-like inclusions in *Mya arenaria* with germinomas from a Maine oil spill site. *Haliotis* **8**, 243–248.

- Hill, B. J. (1976a). Properties of a virus isolated from the bivalve mollusc *Tellina tenuis* (daCosta). In "Wildlife Diseases" (L. A. Page, ed.), Plenum, New York.
- Hill, B. J. (1976b). Molluscan viruses: Their occurrence, culture and relationships. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st pp. 25-29.
- Hill, B. J., and Alderman, D. J. (1979). Observations on the experimental infection of *Ostrea edulis* with two molluscan viruses. *Haliotis* 8, 297-299.
- Hill, B. J., Way, K., and Alderman, D. J. (1986). IPN-like birnaviruses in oysters: Infection or contamination. *Eur. Aquacult. Soc., Spec. Publ.* 9, 297.
- His, E. (1969). Recherche d'un test permettant de comparer l'activité respiratoire des huîtres au cours de l'évolution de la maladie des branchies. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* 33, 171-175.
- Hoover, K. L. (1977). "The Effect of a Virus Infection on the Hemocyte Population in *Carcinus maenas*," Sc.D. thesis. Johns Hopkins Univ., Baltimore, Maryland.
- Hoover, K. L., and Bang, F. B. (1976). Histopathological effects of a virus infection in the shore crab, *Carcinus maenas*. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st pp. 310-311.
- Hoover, K. L., and Bang, F. B. (1978). Immune mechanisms and disease response in a virus disease of *Carcinus*. In "Viruses and Environment" (E. Kurstak and K. Maramorosch, eds.), pp. 515-525. Academic Press, New York.
- Jahromi, S. S. (1977). Occurrence of rhabdovirus-like particles in the blue crab, *Callinectes sapidus*. *J. Gen. Virol.* 36, 485-494.
- Johnson, P. T. (1976a). A herpeslike virus from the blue crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* 27, 419-420.
- Johnson, P. T. (1976b). A baculovirus from the blue crab, *Callinectes sapidus*. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st, p. 24.
- Johnson, P. T. (1976c). An unusual microorganism from the blue crab, *Callinectes sapidus*. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st p. 316.
- Johnson, P. T. (1977a). A viral disease of the blue crab, *Callinectes sapidus*: Histopathology and differential diagnosis. *J. Invertebr. Pathol.* 29, 201-209.
- Johnson, P. T. (1977b). Reovirus-like virus of blue crabs. In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann, ed.), pp. 103-105. Elsevier, New York.
- Johnson, P. T. (1978a). Viral diseases of the blue crab, *Callinectes sapidus*. *Mar. Fish. Rev.* 40, 13-15.
- Johnson, P. T. (1978b). New information on viral diseases of the blue crab, *Callinectes sapidus*. *Proc. Int. Colloq. Invertebr. Pathol.*, 2nd, pp. 85-86.
- Johnson, P. T. (1983). Diseases caused by viruses, rickettsiae, bacteria, and fungi. In "The Biology of Crustacea" (A. J. Provenzano, Jr., ed.), Vol. 6, pp. 1-78. Academic Press, New York.
- Johnson, P. T. (1984). Virus diseases of marine invertebrates. *Helgol. Meeresunters.* 37, 65-98.
- Johnson, P. T. (1988). Development and morphology of an unusual nuclear virus of the blue crab *Callinectes sapidus*. *Dis. Aquat. Org.* 4, 67-75.
- Johnson, P. T., and Bodammer, J. E. (1975). A disease of the blue crab, *Callinectes sapidus*, of possible viral etiology. *J. Invertebr. Pathol.* 26, 141-143.
- Johnson, P. T., and Farley, C. A. (1980). A new enveloped virus from the blue crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* 35, 90-92.
- Koepp, S. J. (1984). Detection of a DNA virus within an upper New York Bay soft-shell clam population. *Coastal Ocean Assessment News* 3(2): 26-28.
- Lauckner, G. (1983). Diseases of Mollusca: Bivalvia. In "Diseases of Marine Animals" (O. Kinner, ed.), Vol. 2, pp. 477-961. Biol. Anst. Helgol., Hamburg, FRG.

- Leibovitz, L., Elston, R., Lipovsky, V. P. and Donaldson, J. (1978). A new disease of larval Pacific oysters (*Crassostrea gigas*). *Proc. Annu. Meet. World Maricult. Soc.*, 9th pp. 603–615.
- Lightner, D. V. (1983). Diseases of cultured penaeid shrimp. In "Mariculture" (J. P. McVey, ed.), pp. 289–320. CRC Press, Boca Raton, Florida.
- Lightner, D. V. (1988a). Reo-like virus (REO) disease of penaeid shrimp. In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann and D. V. Lightner, eds.), pp. 33–37. Elsevier, Amsterdam.
- Lightner, D. V. (1988b). Gut and nerve syndrome (GNS) of *Penaeus japonicus*. In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann and D. V. Lightner, eds.), pp. 104–107. Elsevier, Amsterdam.
- Lightner, D. V., and Redman, R. M. (1981). A baculovirus-caused disease of the penaeid shrimp, *Penaeus monodon*. *J. Invertebr. Pathol.* **38**, 299–302.
- Lightner, D. V., and Redman, R. M. (1985). A parvo-like virus disease of penaeid shrimp. *J. Invertebr. Pathol.* **45**, 47–53.
- Lightner, D. V., Redman, R. M., and Bell, T. A. (1983a). Observations on the geographic distribution, pathogenesis and morphology of the baculovirus from *Penaeus monodon* Fabricius. *Aquaculture* **32**, 209–233.
- Lightner, D. V., Redman, R. M., and Bell, T. A. (1983b). Infectious hypodermal and hematopoietic necrosis, a newly recognized virus disease of penaeid shrimp. *J. Invertebr. Pathol.* **42**, 62–70.
- Lightner, D. V., Redman, R. M., Bell, T. A., and Brock, J. A. (1983c). Detection of IHNN virus in *Penaeus stylirostris* and *P. vannamei* imported into Hawaii from South and Central America. *J. World Maricult. Soc.* **14**, 212–225.
- Lightner, D. V., Redman, R. M., Bell, T. A., and Brock, J. A. (1984). An idiopathic proliferative disease syndrome of the midgut and ventral nerve in the Kuruma prawn *Penaeus japonicus* Bate, cultured in Hawaii. *J. Fish Dis.* **7**, 183–191.
- Lightner, D. V., Redman, R. M., and Bell, T. A. (1985). Histopathology and diagnostic methods for IHNN and MBV diseases in cultured penaeid shrimp. *Proc. Conf. Warm Water Fish Culture—Crustacea*, 1st 23 pp.
- Mari, J., and Bonami, J.-R. (1986). Les infections virales du crabe *Carcinus mediterraneus* Czerniawski, 1884. *Eur. Aquacult. Soc., Spec. Publ.* **9**, 283–293.
- Marteil, L. (1968). La maladie des branchies. *Int. Counc. Explor. Sea, Doc.* **C.M.1968/K**, 5.
- Marteil, L. (1969). La maladie des branchies des huîtres portugaises des côtes françaises de l'Atlantique. Données générales sur la maladie des branchies. *Rev. Trav. Inst. Sci. Tech. Peches Marit.* **33**, 145–150.
- Marteil, L. (1971). Les mortalités d'huîtres en 1970. *Rev. Trav. Inst. Sci. Tech. Peches Marit.* **35**, 227–228.
- Marteil, L. (1976). La conchyliculture française. 2^e partie. Biologie de l'huître et de la moule. *Rev. Trav. Inst. Sci. Tech. Peches Marit.* **40**, 149–345.
- Meyers, T. R. (1979). A reo-like virus isolated from juvenile American oysters (*Crassostrea virginica*). *J. Gen. Virol.* **43**, 203–212.
- Meyers, T. R. (1981). Endemic diseases of cultured shellfish of Long Island, New York: Adult and juvenile American oysters (*Crassostrea virginica*) and hard clams (*Mercenaria mercenaria*). *Aquaculture* **22**, 305–330.
- Meyers, T. R., and Hirai, K. (1980). Morphology of a reo-like virus isolated from juvenile American oysters, *Crassostrea virginica*. *J. Gen. Virol.* **46**, 249–253.
- Oprandy, J. J., and Chang, P. W. (1983). 5-Bromodeoxyuridine induction of hematopoietic neoplasia and retrovirus inactivation in the soft-shell clam, *Mya arenaria*. *J. Invertebr. Pathol.* **42**, 196–206.
- Oprandy, J. J., Chang, P. W., Pronovost, A. D., Cooper, K. R., Brown, R. S., and Yates, V.

- J. (1981). Isolation of a viral agent causing hematopoietic neoplasia in the soft-shell clam, *Mya arenaria*. *J. Invertebr. Pathol.* **38**, 45–51.
- Pappalardo, R., and Bonami, J.-R. (1979). Infection des Crustacés marins due a un virus de type nouveau apparenté aux *Baculovirus*. *C. R. Hebd. Seances Acad. Sci., Ser. D* **288**, 535–537.
- Payen, G. G., and Bonami, J.-R. (1979). Mise en evidence de particules d'allure virale associées aux noyaux des cellules mesodermiques de la zone germinative testiculaire du crabe *Rithropanopeus harrisi* (Gould) (Brachyoure, Xanthidae). *Rev. Trav. Inst. Pêches Marit.* **43**, 361–365.
- Rivers, T. M. (1937). Viruses and Koch's postulates. *J. Bacteriol.* **33**, 1–12.
- Rosenfield, A. (1976). Infectious diseases in commercial shellfish on the middle Atlantic coast. *Spec. Symp. Am. Soc. Limnol. Oceanogr.* **2**, 414–423.
- Rungger, D., Rastelli, M., Braendle, E., and Malsberger, R. G. (1971). A viruslike particle associated with lesions in the muscles of *Octopus vulgaris*. *J. Invertebr. Pathol.* **17**, 72–80.
- Sano, T., Nishimura, T., Oguma, K., Momoyama, K., and Takeno, N. (1981). Baculovirus infection of cultured Kuruma shrimp, *Penaeus japonicus*, in Japan. *Gyobyo Kenkyu* **15**, 185–191.
- Sindermann, C. J. (1976). Oyster mortalities and their control. *FAO Conf. Aquacult. AQ/Conf/76/R.34*, 1–25.
- Sparks, A. K. (1985). "Synopsis of Invertebrate Pathology Exclusive of Insects," 423 pp. Elsevier, Amsterdam.
- Sparks, A. K., and Morado, J. F. (1985). A preliminary report on the diseases of Alaska king crabs. *Proc. Int. King Crab Symp. Lowell Wakefield Fish., Symp. Ser. Alaska Sea Grant Prog.* 10 pp.
- Sparks, A. K., and Morado, J. F. (1986). A herpes-like virus disease in the blue king crab *Paralithodes platypus*. *Dis. Aquat. Org.* **1**, 115–122.
- Tsing, A., and Bonami, J.-R. (1986). A new virus disease in the shrimp *Penaeus japonicus*. *Eur. Aquacult. Soc., Spec. Publ.* **9**, 295.
- Tsing, A., Lightner, D. V., Bonami, J.-R. and Redman, R. M. (1985). Is "gut and nerve syndrome" (GNS) of viral origin in the tiger shrimp *Penaeus japonicus* Bate? *Abstr. Int. Conf. EAEP, 2nd p.* 91.
- Underwood, B. O., Smale, C. J., Brown, F., and Hill, B. J. (1977). Relationship of a virus from *Tellina tenuis* to infectious pancreatic necrosis virus. *J. Gen. Virol.* **36**, 93–109.
- Vago, C. (1966). A virus disease in Crustacea. *Nature (London)* **209**, 1290.
- Yudin, A. I., and Clark, W. H., Jr. (1978). Two viruslike particles found in the ecdysial gland of the blue crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* **32**, 219–221.
- Yudin, A. I., and Clark, W. H., Jr. (1979). A description of rhabdovirus-like particles in the mandibular gland of the blue crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* **33**, 133–147.
- Zerbib, C., Andrieux, N., and Berreur-Bonnenfant, J. (1975). Données préliminaires sur l'ultrastructure de la glande de mue (Organe Y) chez le crabe *Carcinus mediterraneus* sain et parasité par *Sacculina carcini*. *C. R. Hebd. Seances Acad. Sci., Ser. D* **281**, 1167–1169.

3

Bacteria

I. INTRODUCTION

Bacteria are, of course, ubiquitous in the marine environment, so it is not surprising that some will be facultatively pathogenic to weakened animals, and a few may be severe primary pathogens. Short generation times of bacteria assure that massive populations may develop rapidly in infected hosts as well as in the hosts' environment. Population explosions of opportunistic pathogens such as vibrios are especially apparent in confined habitats such as aquaculture tanks or ponds, and it is in such environments that bacterial diseases can be important to the survival of shellfish.

This principal mechanism of disease impact on molluscan and crustacean shellfish populations does not exclude a potential role for virulent primary pathogens, which are capable of sweeping through captive or even wild stocks of shellfish. The best example of such an agent in marine shellfish is the bacterium *Aerococcus viridans* var. *homari*, recognized a half-century ago as the cause of mortalities in natural populations and in captive lobsters on the east coast of North America. The pathogen is probably second only to *Aphanomyces astaci*, a fungal parasite of European freshwater crayfish, in causing major mortalities of crustaceans.

This chapter attempts to summarize information on bacterial diseases of economically important species of marine molluscs and crustaceans—particularly the more recent reports of diseases in aquaculture facilities, where effects at all life cycle stages are visible and sometimes dramatic, and where a whole new understanding of larval diseases is developing.

II. BACTERIAL DISEASES OF MOLLUSCS

Bacterial diseases of molluscs are important in early life history stages, but seem relatively insignificant in populations of adult animals (with a

few notable exceptions). This may be due in part to the facts that the primary defense mechanisms of molluscs—phagocytosis and encapsulation—are particularly competent to deal with pathogens of small size [as was suggested by Sparks (1981)] and that resistance may be age related.

Much of the information about bacterial diseases of molluscs has come from studies of captive or cultured populations; epizootics are usually seen in larval or early juvenile stages. Adult oysters, for example, are less likely to be found with bacterial disease, and, experimentally, they can tolerate exposure to much higher populations of bacteria. In one series of studies, adult oysters, *Crassostrea virginica*, and four other bivalve species exposed to high densities of *Aeromonas* and *Vibrio* were unaffected, while lower concentrations produced disease in larvae (Guillard, 1959; Tubiash *et al.*, 1965). In another study adult mussels exposed to *Vibrio anguillarum* were unaffected, while larvae died, even at the low concentration levels used in the experiments (Martin, 1976).

Vibriosis, sometimes called bacillary necrosis, is undoubtedly the most important disease of bivalve larvae in culture environments. First described in *C. virginica* and in four other bivalves from Connecticut (Tubiash *et al.*, 1965), many aspects of the disease have been examined at the Milford (Connecticut) Laboratory of the National Marine Fisheries Service (Murchelano and Brown, 1968, 1969; Murchelano and Bishop, 1969; Tubiash *et al.*, 1970; Brown and Losee, 1978). Seasonal changes in the abundance of vibrios and other related genera were reported, an exotoxin produced by a *Vibrio* sp. killed developing oyster larvae, bacterial "swarming" around mantle margins of larvae was a reliable indicator of epizootics, and larval mortalities could reach 100%. Studies of vibriosis of oyster larvae in other facilities have been productive as well. The course of disease and subsequent mortalities in a Long Island, New York, oyster hatchery were described by Leibovitz (1978c, 1979), Elston and Leibovitz (1980), and Leibovitz and Elston (1980). Persistent mortalities, attributed to *V. anguillarum* infections of *Crassostrea gigas* larvae, were reported by DiSalvo *et al.* (1978). Bacillary necrosis was also identified as the principal disease problem in a bay scallop (*Argopecten irradians*) culture in Virginia (Castagna, 1975).

Outbreaks of vibriosis in oyster larvae, with associated mortalities, were correlated with peaks in abundance of *Vibrio* in inflow water and in culture water (Leibovitz, 1978a,b). This suggests that bacteriological monitoring must be an important continuing commitment of culture practice if outbreaks are to be predicted and controlled. However, it should be noted that outbreaks of vibriosis in hatcheries may result from strains of pathogens which represent a very small proportion of the total

bacterial population in the seawater system. Brown (1981) described an outbreak in *C. virginica* larval populations in which the disease could be initiated experimentally by adding isolates of the pathogenic strains at dosages of only two cells per milliliter after each change of culture water. The isolates were clearly of highly pathogenic strains of *Vibrio* sp. In a related study at another hatchery, the course of spontaneous infection of oyster larvae by a *Vibrio* sp. was followed, and again the causative organism was found in very low concentrations in the culture water (Brown and Losee, 1978).

Bacterial genera other than *Vibrio* have been implicated in bivalve larval mortalities in hatcheries. An outbreak, with subsequent mortalities of *Ostrea edulis* larvae in the experimental hatchery at Conwy, Wales, was attributed to a highly pathogenic strain of *Pseudomonas* sp. (Helm and Smith, 1971). The course of the disease resembled that of bacillary necrosis of *C. virginica* larvae, as described by Tubiash *et al.* (1965)—even in the swarming of bacteria around moribund animals. Larvae of Pacific oysters, *C. gigas*, were susceptible to the disease, but juvenile *O. edulis* were not.

Effects of bacteria may vary with the stage of larval development or with the species of bivalve larvae being tested. Thus, in one study by Brown (1973), using many isolates of *Vibrio* and *Pseudomonas*, 20 were pathogenic to oyster (*C. virginica*) embryos, but only 12 affected pre-setting larvae. In other studies (Lauckner, 1972; Loosanoff, 1974) larvae of different bivalve species varied in susceptibility to bacterial infections from known isolates.

Additionally, effects on larvae may vary with the particular strains or isolates of bacteria used in experimental exposures. Observed effects in one study included abnormal embryonic development (leading to incomplete shell formation or velum protrusion), decreased growth, or death of larvae when the veliger state was reached (Brown, 1973). Forty-eight isolates of *Vibrio* and *Pseudomonas* were used in the experiments; some caused developmental abnormalities, some reduced growth, and some caused mortalities. In another study (Elston and Leibovitz, 1980) three types of pathogenesis were described: (1) progressive mantle disruption, (2) severe velar deformation and damage, and (3) progressive visceral lesions and atrophy. The patterns of disease seemed related to the isolate used for inoculation.

The effects of vibrios and other bacteria on wild populations of bivalve molluscs are less well known, but some information exists. Based on experimental studies, Tubiash *et al.* (1965, 1970) suggested that natural epizootics could occur among oyster larvae, and that this could reduce year class survival—but evidence for this does not exist. Effects

of *Vibrio* infections on adult oysters have been observed, however. *Vibrio*-induced cardiac edema was reported in low prevalence in oysters from Chesapeake Bay (Tubiash *et al.*, 1973). Isolates of pericardial fluids contained high densities of *V. anguillarum*, which proved to be pathogenic experimentally to oyster larvae but not to adults.

Reports of mass mortalities of possible bacterial etiology in Pacific oysters, *C. gigas*, have been published in Japan (Fujita *et al.*, 1953, 1955; Kan-no *et al.*, 1965; Mori *et al.*, 1965a,b; Numachi *et al.*, 1965; Tamate *et al.*, 1965). Tukeuchi *et al.* (1960) implicated a gram-negative, motile, 1- to 3- μ m bacillus, probably an *Achromobacter*, in large-scale mortalities in oyster culture areas of Hiroshima Bay beginning in 1946. Experimental infections were achieved with cultured bacteria, but the organisms could be isolated from healthy as well as sick oysters and from seawater. Moribund oysters had diffuse cell infiltration, a massive increase in bacterial numbers, and tissue necrosis.

Numachi *et al.* (1965) found up to 20% infection with gram-positive bacteria (not further identified) in oysters during mass mortalities in Matsushima Bay, Japan, in the early 1960s. Called "summer disease," it was characterized by the presence of multiple abscesses—areas of focal necrosis in which bacteria could be found—but the authors did not think that a causal relationship existed between bacteria and mortalities. A similar disease was found in 1965 by staff members of the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) Biological Laboratory, Oxford, Maryland, in seed oysters (less than 1 year old) imported to the United States West Coast from Matsushima Bay, and in adult oysters from Willapa Bay, Washington. The disease has been labeled "focal necrosis" (Fig. 5); it affects seed oysters as young as 6 months, as well as adults. The necrotic foci or multiple abscesses may represent the resistant or arrested disease state, and the active, fulminating phase may have already killed susceptible members of the population early in their existence (Sindermann and Rosenfield, 1967).

Mortalities of *C. gigas* on the Pacific coast of North America have occurred sporadically since the early 1960s, as they have in Japan, and uncertainty about their etiology still prevails—except that vibrios and other bacteria seem to be implicated. *Pseudomonas enalia* was isolated from dying oysters in Puget Sound, and injected cultured organisms reproduced the disease and caused mortalities (Colwell and Sparks, 1967). In other studies oysters transferred from commercial beds to laboratory tanks died, and *Vibrio parahaemolyticus* was a consistent isolate (Lipovsky and Chew, 1971). Focal lesions and hemocyte infiltration were seen, and mortality was directly related to temperature.

In subsequent studies high-temperature stress, accompanied by in-

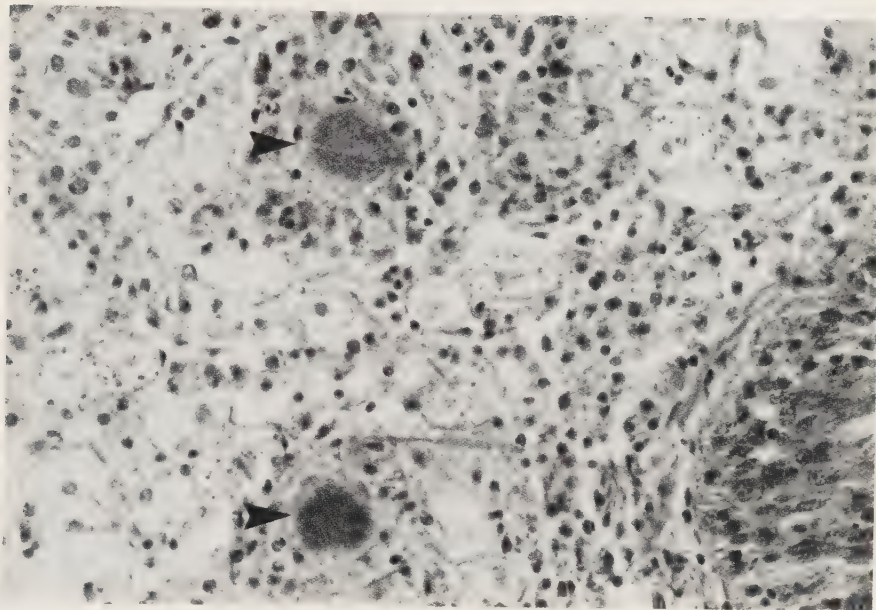


Figure 5. Focal necrosis in connective tissue of Pacific oyster from Willapa Bay, Washington, surrounded by extensive hemocyte infiltration.

fection with opportunistic bacteria, was blamed for continuing mortalities of *C. gigas* in the Pacific Northwest. Losses due to summer disease have been attributed to *Vibrio* infections and high water temperatures (Lipovsky and Chew, 1971, 1972, 1973; Grischkowsky and Liston, 1974; Lipp *et al.*, 1976). Annual mortalities were observed in Puget Sound populations, and vibrios were isolated from body fluids. Mortalities were produced in experimental populations following exposure to vibrios at temperatures above 18°C. In a related study, oyster mortalities in British Columbian waters occurred in summers with higher than normal temperatures (Quayle, 1969).

The general conclusion about recent mass mortalities of Pacific oysters, whether they occur in Japan or in North America, is that the summer disease is probably of bacterial etiology, possibly aggravated by physiological stresses of spawning and high water temperatures—a conclusion also reached by Sparks (1981).

Attempts to elucidate effects of bacteria on bivalve molluscs often, then, represent exercises in frustration. Contributing to uncertainties are these factors:

- The taxonomy of the important facultatively pathogenic groups—especially *Vibrio*, *Pseudomonas*, *Aeromonas*—is extremely disorganized.
- Pathogens are part of the normal marine flora and may be found in the habitat, the digestive tract, and even the tissues of presumably healthy animals.
- Earlier identifications of molluscan pathogens may be modified as new diagnostic tests are developed.
- Vibrios and other bacterial groups consist of numerous strains, biovars, and biotypes, which seem to be evolving rapidly (Regal, 1986) and which may vary widely in their pathogenicity for any host species (Jeffries, 1982).
- Bacteria pathogenic to bivalve larvae in culture environments may or may not be pathogenic in natural populations; cultured larvae may appear more susceptible, since they are reared in static systems with elevated temperatures and at high population densities (Brown, 1973).

Despite these limitations, some general principles are beginning to emerge:

- Bacterial diseases of molluscs and other invertebrates are usually caused by species of bacteria which (1) are able to survive phagocytosis, (2) have developed mechanisms to escape phagocytosis, or (3) can overwhelm stressed hosts (Sparks, 1981).
- Bacterial diseases of larvae in *natural* populations are not well understood, but, extrapolating from observed effects of bacterial diseases of *cultured* larvae, they may be severe. Thus, it may be that a disease such as focal necrosis of Pacific oysters (*C. gigas*), which appears somewhat benign in adult oysters, may have severe effects on larvae or early juveniles.
- Temperature influences on the occurrence and the severity of infections have been demonstrated in several bivalve species; the extent to which temperature affects abundance of the pathogen population in the aquatic environment may be a determining factor in outbreaks.
- Experimental infections achieved by exposure of bivalves (or other marine animals, for that matter) to massive doses of presumed pathogens—whether by inoculation or by loading a restricted experimental environment—may not provide accurate information on pathogenicity under less artificial conditions. The relatively nonspecific resistance mechanisms of molluscs may be overwhelmed by such abnormally high dosages, whereas natu-

rally occurring population levels of facultative organisms may be dealt with successfully by the normal animal.

- Bivalves live in an environment rich in bacteria, and their filter feeding results in ingestion of many kinds of microorganisms—including those such as vibrios and pseudomonads, which are potentially pathogenic. Furthermore, bacteria can be found frequently in the tissues, even though cellular and humoral defenses exist.
- Disease caused by facultative pathogens may be a consequence of increased infection pressure and/or decreased resistance to tissue invasion—the former often resulting from environmental conditions favorable to reproduction of facultative microorganisms, and the latter a possible consequence of environmental stressors.
- Pathogenicity of specific strains of *Vibrio* and *Pseudomonas* may be important in disease outbreaks among bivalve larvae in hatcheries—so that virulent organisms representing a tiny proportion of the bacterial population in culture water may cause mass mortalities.

Among molluscs other than bivalves, a few severe bacterial diseases have been reported from gastropods and cephalopods being reared for purposes of experimentation or food production. In the development of intensive culture systems for red abalone, *Haliotis rufescens*, continuous background mortalities, with occasional mass mortalities of juveniles, have been attributed to *Vibrio* infections, often in association with or provoked by environmental stressors such as high oxygen saturation and high temperature (Elston and Lockwood, 1983). Characteristics of the most common pathogenic isolates corresponded most closely to those of *Vibrio alginolyticus*; muscular and connective tissues of the foot were most seriously involved during the active phase of invasion; and mortalities ensued. The disease was considered irreversible at an early stage, and little evidence of host response was seen. Proposed control measures included a reduction in pathogen numbers in the system and the control of predisposing stress factors.

Vibrio infections, associated with skin lesions and penetrating ulcers, have been examined in cephalopods. A fatal ulcerative condition was reported in young (1- to 2-month-old) octopuses, *Octopus joubini* and *Octopus briareus*, reared in high-density systems (Hanlon *et al.*, 1984). Bacteria of several genera were isolated from ulcers; the disease was reproduced experimentally by isolates resembling *V. alginolyticus*; and ulcers appeared within 2 days after exposure. The disease was

controlled by nifurpirinol dips followed by isolation of ulcerated specimens. The authors concluded that the condition was a consequence of crowding and increased contact among individuals. In other studies vibrios have been isolated from lesions in captive squids, *Loligo pealei* (Leibovitz *et al.*, 1977; Hulet *et al.*, 1979).

III. BACTERIAL DISEASES OF CRUSTACEANS

The major bacterial diseases of Crustacea have been investigated for more than half a century, and the literature is extensive and expanding. Three diseases (or disease complexes) have received most of the research attention: (1) shell disease, caused by chitinolytic microorganisms, (2) gaffkaemia of lobsters, caused by *Aerococcus viridans* var. *homari*, and (3) vibriosis, caused by several species of *Vibrio*. Shell disease and vibriosis seem to be consequences of interactions of facultative pathogens and environmental stressors, whereas gaffkaemia is clearly the result of invasion by a virulent primary pathogen. Each of these diseases (plus some material on bacterial epibionts) is considered in some detail in this section, with emphasis on the effects on survival of infected animals.

A. Shell Disease

Probably the most common and widespread bacterial disease of crustaceans is that which causes various kinds of exoskeletal erosions, due to the action of chitinoclastic bacteria (and possibly other chitin-destroying microorganisms as well). Bacteria of this type are common in the sea (Benton, 1935; Zobell and Rittenberg, 1938; Hock, 1940, 1941; Lear, 1963; Hood and Meyers, 1974). They usually degrade the exoskeletons of dead animals but may also affect living individuals. Over 30 species of chitin-destroying bacteria are known, of which half have been isolated from the shells of crustaceans.

King crabs, *Paralithodes camtschatica* and *Paralithodes platypus*, from the eastern North Pacific are occasionally affected by rust disease, which seems to result from the action of chitin-destroying bacteria on the exoskeleton. Bright *et al.* (1960)⁴ described observations of rust disease in landed catches of king crabs from Kachemak Bay, Cook Inlet, Alaska, as

⁴Data were furnished from an unpublished contract report, *King Crab Investigations of Cook Inlet, Alaska*, by Donald B. Bright, Floyd E. Durham, and Jens W. Knudsen of the Allan Hancock Foundation, University of Southern California, Los Angeles, to the U.S. Bureau of Commercial Fisheries Biological Laboratory, Auke Bay, Alaska, June 1960. (Cited with permission—dated 1969—of the Laboratory Director, U.S. Bureau of Commercial Fisheries Biological Laboratory, Auke Bay, Alaska.)

well as experimental studies of the bacteria involved. The disease was characterized by progressive darkening and softening of the exoskeleton, particularly on the ventral surfaces. Underlying living tissues were unaffected. Natural infections reached 11% in larger, older crabs in 1957 but were much lower in 1958 and 1959. Shell abrasions and injuries served as foci of the disease, which developed experimentally within 2 weeks. The disease was not carried over to the new exoskeleton after molting, but recently shed crabs were highly susceptible because the new shell was easily punctured or abraded. Chitin-destroying bacteria were isolated from infected crabs, and cultured organisms produced the disease experimentally in normal crabs. Similar bacteria were also isolated from seawater in Kachemak Bay. The authors concluded that the disease would not affect the commercial fishery seriously unless the catch of crabs was substantially less than annual recruitment, since larger individuals, which do not molt annually, were more frequently infected. Since that early report by Bright *et al.*, similar exoskeletal lesions have been observed in tanner crabs, *Chionoecetes tanneri*, with prevalences up to 76% along the Pacific Northwest coast of the United States (Baross *et al.*, 1978).

A condition known as *Brandfleckenkrankheit*, or burn spot disease, occurs in European edible crabs, *Cancer pagurus* (Schäfer, 1954; Gordon, 1966). Although it is thought by some to be of fungal etiology, others believe that it may be due to chitinovorous bacteria. Gross pathology consists of dark brown to black spots with red margins on the carapace and appendages, particularly the chelipeds. The chitin in the center of the spots becomes friable and may be destroyed, exposing the underlying musculature. Gordon reported that in 1956 many diseased crabs from waters to the southwest of England and from the North Sea reached the market. A similar disease is known in European freshwater crayfish and river crabs, from which three fungi have been isolated and described (Mann and Pieplow, 1938; Mann, 1939).

An exoskeletal disease, caused by chitin-destroying microorganisms, was described by Rosen (1967) in blue crabs from Chesapeake Bay. Chitinoclastic bacteria were isolated from characteristic brown depressed areas of chitin destruction. The disease was progressive and was evident in 3% of the crabs examined. It is important to note that the crabs had been crowded together in shedding tanks for an extended period. Shell disease has also been seen in blue crabs from Galveston Bay, Texas (S. H. Hopkins, personal communication, 1968), the Mississippi coast (Overstreet, 1978), Biscayne Bay, Florida (Iversen and Beardsley, 1976), the South Carolina coast (Sandifer and Eldridge, 1974), and the Pamlico River, North Carolina (Engel and Noga, 1989) (Fig. 6).

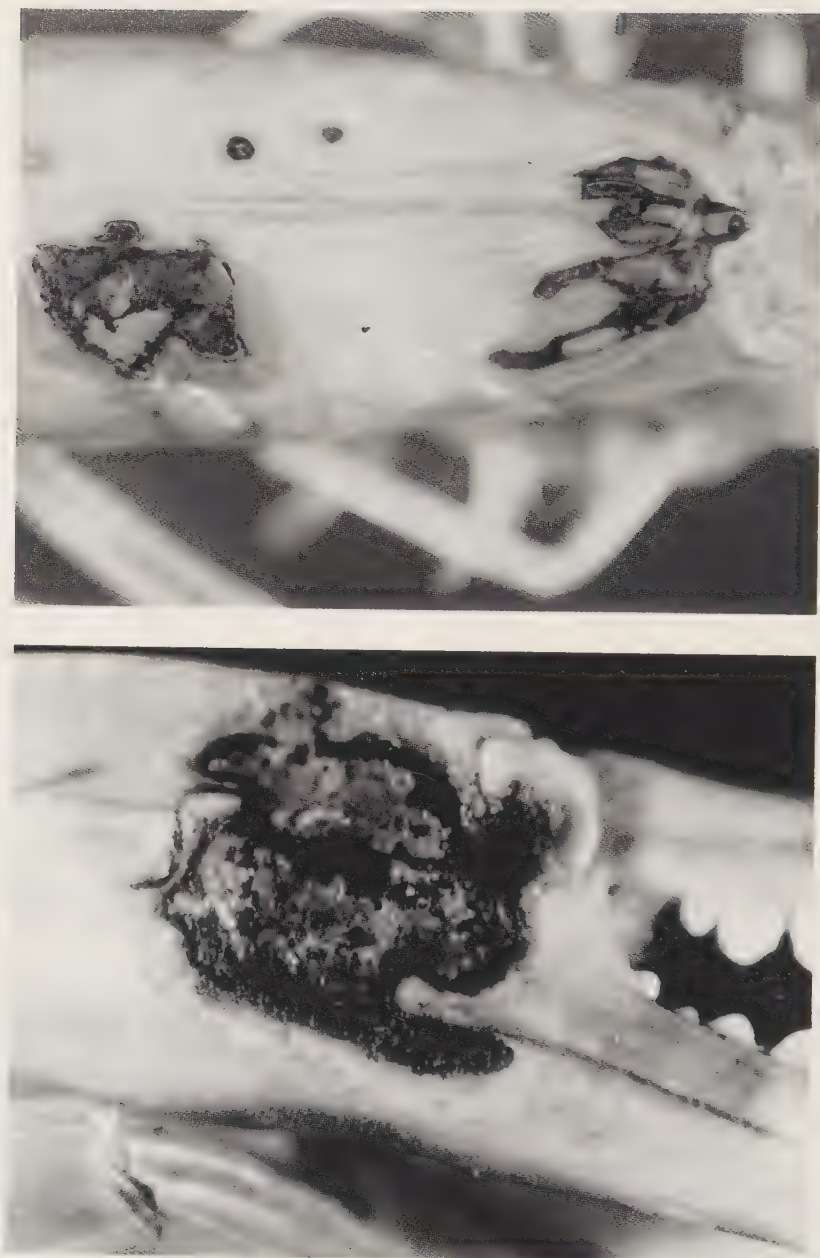


Figure 6. Advanced shell disease in brown shrimp carapace (top) and blue crab claw (bottom). (Courtesy of Dr. R. M. Overstreet.)



Figure 7. Shell disease of lobsters caused by chitin-destroying bacteria.

Still another shell disease, of possible bacterial etiology, in English prawns, *Palaemon serratus*, was described briefly by Anderson and Conroy (1968). Labeled "brown spot disease" and characterized by erosion and destruction of the exoskeleton, the disease was particularly evident in crowded culture tanks. Although the etiology was undetermined, the authors observed an association between the occurrence of spots and the presence of myxobacteria. A similar brown spot condition was seen in penaeid shrimp from the Gulf of Mexico (Cook and Lofton, 1973; Iversen and Beardsley, 1976; Cipriani *et al.*, 1980) (Fig. 6).

Shell disease caused by chitinolytic bacteria is also found in American lobsters, *Homarus americanus*. Hess (1937) first isolated chitin-degrading bacteria from live lobsters impounded at Yarmouth, Nova Scotia, but collected from various parts of the Canadian Maritime provinces. This was the first report of attacks by such microorganisms on living Crustacea. The disease was characterized by pitting and sculpturing of the exoskeleton (Fig. 7); although it was first seen in impounded lobsters, similar conditions were later observed in freshly caught lobsters from several widely separated Canadian fishing grounds. Initial lesions occurred on the walking legs and were distinguished by white outer mar-

gins, from which the bacteria were most readily isolated. Hess found the disease to be relatively rare in natural populations but noted severe shell erosion and weakening of lobsters stored in pounds over the winter. Isolated microorganisms were able to decompose pure chitin in saline solution containing no other nitrogen or carbon source. None of Hess' isolates—nor, for that matter, isolates prepared in subsequent work with lobsters—was reported to reproduce the disease experimentally.

Significant mortalities of lobsters accompanied the shell disease; Taylor (1948, 1949) found that 71% of the infected captive lobsters died from the disease, but observed no correlation between mortality and the intensity of external shell erosion. Acquisition of the disease by healthy lobsters placed in seawater tanks with infected individuals indicated direct transmission. The disease developed slowly, requiring at least 3 months before the advanced stages were reached. Progress of chitin destruction was directly temperature dependent and new shell laid down after molting was not affected, except by reinfection (McLeese, 1965).

Sawyer and Taylor (1949) observed that shell disease produced thickening or complete destruction of the chitinous layer of the gill filaments. No living gill tissue was attacked, but the authors postulated respiratory impairment as an important consequence of the disease. The infection appeared to be entirely external, confined to the exoskeleton; it did not invade living tissue nor was it transmitted internally. Sawyer and Taylor also reported the disease to be present on the Maine coast as well as in Canada and considered it a potential threat to the lobster industry, in view of the ease of transmission and the observed mortalities of captive individuals. The method of infection of lobsters was unknown; lodging of bacteria in pores and ducts of the shell was proposed by Sawyer and Taylor as a route of invasion.

Since those early, detailed studies of shell disease in lobsters, a number of additional aspects have been investigated. Two studies—one of *H. americanus* in the New York Bight and the other of *H. gammarus* in the Oslofjord—have indicated a possible association with degraded habitats (sewage sludge dump sites) (Young and Pearce, 1975; Roald *et al.*, 1981). Also, larval and postlarval lobsters have been found to be susceptible as well as adults (Fisher, 1977; Fisher *et al.*, 1976). The recent studies have frequently implicated *Vibrio* spp. as etiological agents of shell disease of lobsters, although pseudomonads and aeromonads have also been isolated (Malloy, 1978; Roald *et al.*, 1981). Also, mechanical damage to the epicuticle of the shell is considered by some investigators to be essential to invasion by chitinolytic organisms (Rosen, 1970; Fisher *et al.*,

1978), although other routes are possible, such as synergistic activity of lipolytic bacteria (Baross and Tester, 1975).

B. *Gaffkaemia*

Undoubtedly, the most virulent systemic bacterial pathogen found in crustaceans is *Aerococcus viridans* (var. *homari*), a gram-positive, tetrad-forming encapsulated coccus. It is known from wild and impounded populations of American lobsters, *H. americanus*, and European lobsters, *H. gammarus*. The disease (known as "gaffkaemia" because of an earlier designation of the pathogen as a member of the now-defunct bacterial genus *Gaffkya*) was first noted on the Maine coast in 1946 (Hitchner and Snieszko, 1947; Snieszko and Taylor, 1947; Getchell, 1949). Red-tail disease, as it was originally called, is characterized by a variable pink coloration of the ventral abdomen, pink blood, prolonged clotting time, and a drastic reduction in blood phagocytes. Infected lobsters become progressively weaker, and mortalities may reach 50% after short periods of storage. Mortalities increase sharply when water temperature exceeds 15°C. Moribund lobsters move to shoal water and die in a "spread-eagle" position.

Goggins and Hurst (1960)⁵ provided information about two epizootics of gaffkaemia along the entire Maine coast, one in 1946–1947 and another in 1959–1960, with losses as great as 58% of impounded populations. They found that the pathogen could live and multiply outside the lobster in the slime on lobster cars, crates, tanks, and live wells. *Aerococcus viridans* was also isolated from the mud of tidal pounds and from seawater several miles from infected pounds. The disease was transmitted directly, by allowing presumably healthy lobsters to feed on infected individuals or by holding healthy lobsters in seawater containing the pathogen. Incubation time was 14–21 days, although the animals possibly were already gaffkaemic before the start of the experiments. Treatment of tidal ponds with calcium hypochlorite reduced populations of the pathogen in bottom mud and reduced subsequent losses of impounded lobsters.

The disease organism is often present in wild populations. Stewart and MacDonald (1962) and Stewart *et al.* (1966) isolated *A. viridans* from 96 of 2035 recently caught lobsters in Canada and found the disease to be widespread in the Canadian Atlantic region. Cornick and Stewart (1966)

⁵Data were provided from an unpublished mimeographed report of the Department of Sea and Shore Fisheries, Augusta, Maine: *Progress Report on Lobster Gaffkyaremia (Red Tail)*, by P. L. Goggins and J. W. Hurst, 1960.

recovered, from presumptive tests for the pathogen in Canadian lobsters, several other kinds of bacteria, including *Pseudomonas*, *Achromobacter*, and *Brevibacterium*, none of which was considered to be pathogenic. Rabin (1965) found the organism in lobsters from Woods Hole, Massachusetts, and a recent study disclosed prevalences of up to 22% in lobsters from the Maine coast (Vachon *et al.*, 1981). Wood (1965a,b) isolated the pathogen from two North Sea lobsters, *H. gammarus*. He observed mortalities in storage tanks in southern England in 1962 and recovered an agent with cultural and biochemical characteristics similar to Canadian and United State isolates. Epizootics of gaffkaemia have been reported from European lobsters in Ireland (Gibson, 1961), Norway, and The Netherlands (Roskam, 1957). Gibson noted that diseased lobsters were also infected with the gill maggot, *Nicthoë astaci*, which was absent from uninfected individuals.

The etiological agent of gaffkaemia has undergone several taxonomic changes. It was originally designated *Gaffkya homari* by Hitchner and Snieszko (1947). Then Deibel and Niven (1960), examining various cocci from meat-curing brines, humans, and isolates of *G. homari* from lobsters, found them sufficiently similar in morphology and physiology to one another and to members of the genus *Pediococcus* to justify placing them in a single species of *Pediococcus*, which they designated *Pediococcus homari*, nov. comb. Still later, the entire genus *Gaffkya* was suppressed, and the lobster pathogen was designated *Aerococcus viridans* var. *homari* (Kocur and Martinec, 1965; Editorial Secretary, 1971; Kelly and Evans, 1974).

Recent studies have added incrementally but substantially to information about gaffkaemia. Continuing contributions to understanding the disease process have been made by Stewart and associates (Stewart and Cornick, 1967, 1972; Stewart *et al.*, 1969a–d, 1972a,b, 1975; Stewart and Rabin, 1970; Stewart and Zwicker, 1972; Stewart and Arie, 1973a,b, 1974; Cornick and Stewart, 1975; Stewart, 1975, 1978; Johnson *et al.*, 1981). Additionally Stewart (1975, 1980, 1984) has summarized knowledge of the disease periodically. The available literature on this disease far exceeds that for any other crustacean disease (with the possible exception of European crayfish plague).

Physiological aspects of gaffkaemia have been investigated in detail. Temperature is important to the disease process: at 1°C the pathogen survived and retained virulence, but did not multiply; at 4–20°C the mean time to death for inoculated lobsters decreased consistent with increased growth rates of *A. viridans* (Stewart *et al.*, 1969c; Stewart and Rabin, 1970). The size of the initial inoculum had little effect on survival time (Cornick and Stewart, 1968a). The pathogen appear, to lack invas-

ive capability, so transmission requires rupture of the exoskeleton in the presence of the infective agent (Stewart *et al.*, 1969a). Multiplication of *A. viridans* occurs in the hemolymph after an initial concentration in the hepatopancreas and is accompanied by a reduction in the number of circulating hemocytes, prolongation of clotting time, and a drastic reduction of glucose, lactic acid, and nonprotein nitrogen levels in the hemolymph (Stewart *et al.*, 1969d; Stewart and Arie, 1973b). Death results from impairment of hepatopancreatic functions, after the depletion of energy reserves (Stewart, 1980).

Experimental studies of host-parasite relationships showed that lobsters became infected and died a few days after inoculation with *A. viridans* (Rabin, 1965). Prior inoculation with *Vibrio* endotoxin did not enhance the infection, and prior inoculation of heat-killed *A. viridans* did not alter the course of infection. Lobster serum stimulated *in vitro* growth of the organism in almost every test, but growth of *Vibrio* was sometimes inhibited. Studies of possible defense mechanisms of lobsters against *A. viridans* were carried out at the Halifax (Nova Scotia) and St. Andrews (New Brunswick) stations of the Fisheries Research Board of Canada. Lobster serum, as indicated by Rabin's work, had no antibacterial activity against the pathogen, but instead promoted its growth.

Cornick and Stewart (1968a) published an extensive study of the natural defense mechanisms of the lobster against *A. viridans* and other bacteria. Experimental infection with *A. viridans* was almost invariably fatal; even at doses approximating only five organisms per lobster, 90% of the experimental animals were killed in 17 days. This indicated a lack of effective defense against the pathogen. Using four different bacteria, one of which was *A. viridans*, Cornick and Stewart found lobster serum *in vitro* to be bactericidal or bacteriostatic for all but *A. viridans*, whose growth was stimulated. Lobster serum also agglutinated *in vitro* eight different bacteria, but not *A. viridans*. Fluorescence-labeled pathogens were found to be actively phagocytized *in vivo*. Phagocyte numbers were markedly reduced after injections of bacteria, but returned to normal in a few hours. Some evidence for possible inactivation of *A. viridans* was seen in the formation of nodules in the gills containing tightly clumped reddish-brown tissue cells containing the pathogens. That this defense mechanism is not effective is indicated by the fact that low dosages are able to establish fatal infection. The Atlantic rock crab, *Cancer irroratus*, was found to be susceptible to the pathogen by Cornick and Stewart (1968b) and by Fisher *et al.* (1979). Moderate to heavy infections were produced experimentally. The mean time to death was 6 weeks, as opposed to less than 3 weeks for lobsters. The authors believed that the activity of serum agglutinins of the crab might account in part for the

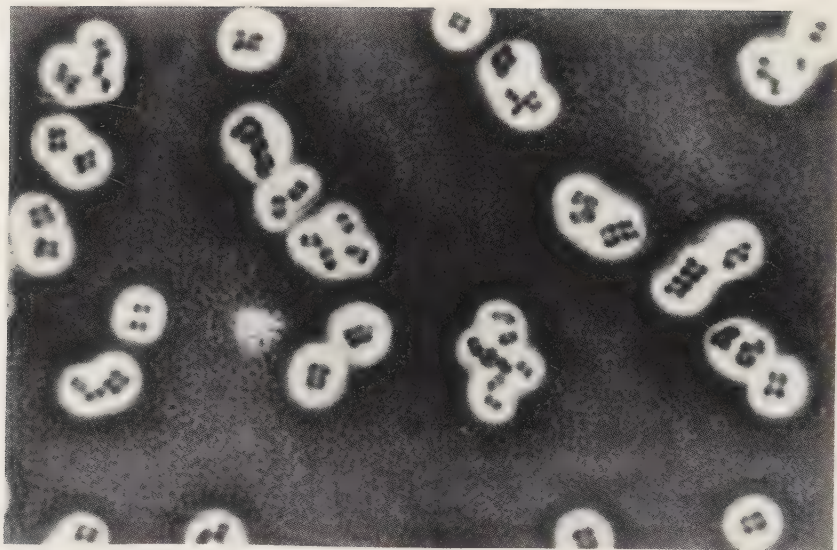


Figure 8. Hemolymph smear of lobster, *Homarus americanus*, showing numerous encapsulated *Aerococcus viridans* var. *homari*.

milder infection, and they suggested that crabs might serve as reservoirs of infection for lobsters. A related study by Stewart and Dingle (1968), comparing sera of the crabs *Cancer irroratus*, *Cancer borealis*, and *Hyas coarctatus*, disclosed no differences that would exclude *A. viridans* as a potential pathogen for *C. borealis* or *H. coarctatus*.

Lobsters, unlike some other crustaceans, thus seem to have no effective natural internal defenses against virulent strains of *A. viridans*; resistance to infections has been demonstrated only rarely (Rabin and Hughes, 1968). Naturally occurring bactericidins and agglutinins are ineffective against the pathogen, probably because of its heavy polysaccharide capsule. Phagocytosis occurs, but the organisms continue to multiply, again probably because of the presence of the capsule, and the phagocytic hemocytes are destroyed (Fig. 8).

Artificial protection by vaccination of lobsters has been attempted, with some success. The phagocytic index of hemocytes and plasma bactericidin levels have been increased by injections of *Pseudomonas perolens*, and high levels of resistance can be induced by injections of virulent *A. viridans* in combination with the antibiotic vancomycin (Stewart and Zwicker, 1974). Injections of heat-killed pathogens have also provided some increase in the survival rate of challenged individuals (Arie and Stewart, 1984).

C. *Vibriosis*

In addition to the chitinoclastic bacteria and the etiological agent of gaffkaemia, other bacteria can be important as pathogens of Crustacea. As might be expected, vibriosis—a universal problem in coastal/ estuarine animal populations—has its manifestations in many crustacean species, and it has been the object of a number of studies. *Vibrio* infections have been observed frequently in cultured penaeid shrimp (Lightner, 1977a). Most isolates are *V. parahaemolyticus*, *V. anguillarum*, or *V. alginolyticus*, although other bacterial genera may be involved occasionally (especially species of *Pseudomonas* and *Aeromonas*). As examples of some of the studies, *V. parahaemolyticus* was identified as a cause of high mortality in pond and tank-held populations of brown shrimp, *Penaeus aztecus*, by Vanderzant *et al.* (1970, 1971). Cultured isolates caused mortalities of normal shrimp within 3 hours when added to experimental aquaria. In a subsequent study (Lewis, 1973) *V. anguillarum* was isolated from moribund brown shrimp from an experimental pond in which mass mortalities had occurred. Isolates were pathogenic to normal adults within 24 hours following injection, with foci of infection in the hepatopancreas.

Noteworthy from an aquaculture perspective was an epizootic of *Vibrio* sp. in cultured Kuruma prawn, *Penaeus japonicus*, in Japan (Takahashi *et al.*, 1985). The infection was systemic, with foci in the heart, lymphoid organ, and skeletal muscles. Gross disease signs were cloudiness of body muscle at the sixth abdominal segment and brown spots in the gills and lymphoid organ.

Important aspects of vibriosis in penaeid shrimp were summarized by Lightner (1983) and by Lightner *et al.* (1984) as follows:

- Infections may be chronic, subacute, or acute, and mortality may reach 100% in some cultured populations.
- Most outbreaks are consequences of extreme stresses and opportunistic pathogens.
- Isolates of *Vibrio* spp. from shrimp may not always produce experimental infection, except when massive doses are injected.
- Larval, postlarval, juvenile, and adult shrimp may be infected.
- *Vibrio* spp. which infect shrimp are ubiquitous, and have been reported from all major shrimp culture regions.
- *Vibrio* species and strains differ markedly in their virulence for penaeids, as they do for other hosts.

Mortalities of several species of crabs have been attributed to vibriosis—especially when the animals are held in captivity. Mortalities

of 50% or higher have been reported in blue crabs, *Callinectes sapidus*, held in commercial shedding facilities (Krantz *et al.*, 1969). *Vibrio parahaemolyticus* was a consistent isolate from moribund crabs, and experimental crabs injected with bacterial cultures from diseased individuals died in 1–3 days (Tubiash and Krantz, 1970).

Examination of vibriosis in wild-caught blue crabs from the Middle Atlantic coast disclosed severe effects within 10 days after capture, leading to the conclusion that most bacterial infections were acquired during capture and transport, from facultative pathogens in the water or adherent to the body surfaces (Colwell *et al.*, 1975; Johnson, 1976).

Vibriosis in rock crabs, *C. irroratus*, was reported in prevalences of 12% in New England waters (Newman and Feng, 1982). The isolates were pathogenic over a wide range of temperatures, and experimental inoculations produced mortalities in less than 24 hours at 20°C.

Presumptive vibriosis has also been reported in the shore crab (*Carcinus maenas*) from the northern European coast (Spindler-Barth, 1976). Hemolymph of infected individuals appeared milky because of the numbers of bacteria present; both acute and chronic infections were produced experimentally by injection, with acute infections lethal to most individuals in 1–6 days.

Mortalities of juvenile lobsters, *H. americanus*, being cultured in California have been attributed to effects of *Vibrio* sp. (Bowser *et al.*, 1981). Isolates from moribund lobsters, when cultured and injected into normal juveniles, caused death within 24 hours. The presence of a bacterial endotoxin was postulated as contributing to the disease process. The organism was also isolated from moribund adult lobsters which had been subjected to experimental dietary deficiencies, suggesting a facultative association of bacterium and host. In other studies *Vibrio* sp., *V. parahaemolyticus*, and *V. alginolyticus* have been isolated from moribund spiny lobsters as well (Brinkley *et al.*, 1976).

Vibriosis is, obviously, an important problem for Crustacea, especially for captive or cultured populations, in which environmental stressors or injuries facilitate invasion and multiplication of facultative pathogens.

D. Epibionts

The bacterial diseases discussed thus far—shell disease, gaffkaemia, and vibriosis—are of greatest significance to wild or cultured crustaceans, but there are other bacteria which occur as epibionts on cultured early life history stages and which may cause mortalities, usually by reducing gas exchange. These fouling organisms, filamentous and nonfilamentous, have caused mortalities of eggs and larvae of penaeid shrimp,

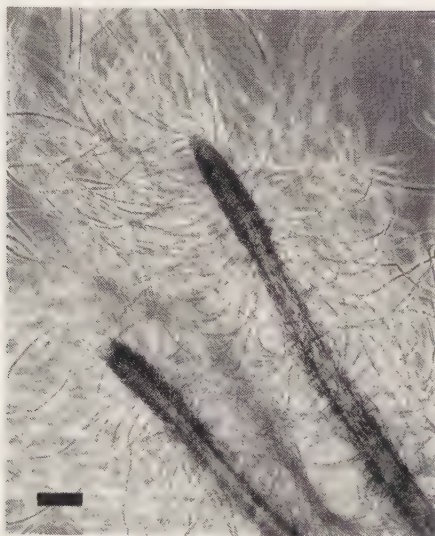


Figure 9. *Leucothrix mucor* filaments on the gill of a juvenile shrimp (*Penaeus californiensis*). Bar, 50 μ m. (Courtesy of Dr. D. V. Lightner.)

crabs, and lobsters (Johnson, 1974; Nilson *et al.*, 1975; Fisher, 1976). *Leucothrix mucor*, a filamentous form, is most commonly implicated (Fig. 9), although a mixed population of nonfilamentous bacteria, algae, and stalked protozoans may also participate (Lightner, 1977b). The extent of fouling is usually a result of poor water quality—especially high nutrient loading (Fisher *et al.*, 1978).

These, then, are the significant bacterial disease problems in marine crustaceans. The available information can be summarized as follows:

- An exoskeletal disease, resulting in shell erosions, is common in Crustacea; chitinolytic bacteria (including *Vibrio* spp.) are frequently isolated from such lesions, although fungi may also be involved.
- Systemic infections of Crustacea by facultative bacterial pathogens may be the consequences of injury—breaching exoskeletal defenses—enhanced by the presence of environmental stressors (Johnson, 1976). Prominent among the opportunistic microbial invaders of stressed crustaceans are several *Vibrio* species, especially *V. parahaemolyticus*, *V. alginolyticus*, and *V. anguillarum*.
- The concept of disease as the outcome of the interaction of facultative pathogen with environmental stressor can be applied to many bacterial diseases of Crustacea, except for gaffkaemia, caused by *A. viridans* var. *homari*, which is a primary and virulent

pathogen of lobsters. Gaffkaemia is highly specific for lobsters but is capable of producing low-grade infections in other crustaceans. Critical elements of the disease process include failure of host internal resistance mechanisms, leading to energy depletion, hepatopancreatic dysfunction, and death.

IV. CONCLUSIONS

Bacterial diseases of molluscan and crustacean shellfish have become much better known as intensive marine aquaculture has developed and as disease problems have emerged in hatcheries and culture tanks. Until recently, though, bacteria were only *assumed* to be pathogenic in natural populations—except for a few crustacean disease agents, such as that causing gaffkaemia in lobsters, recognized earlier in this century.

Among the molluscs, vibriosis has become a dominant factor and a principal disease problem in aquaculture, with severe effects on larvae of oysters, clams, and scallops. Highly pathogenic strains of *Vibrio* can destroy larval populations in a matter of hours; mortalities often reach 100%. Mass mortalities of adult oysters have in some instances been attributed to bacterial infections, but evidence is inconclusive.

In crustaceans, vibriosis is a significant problem, especially in shrimp culture, where larval and juvenile populations may be totally eliminated by outbreaks. Natural and impounded populations of lobsters may be affected by other diseases as well; effects of shell disease and gaffkaemia are both well known for their serious effects. Shell disease is caused by chitinoclastic bacteria and possibly some fungi, while gaffkaemia is caused by a virulent gram-positive coccus, *A. viridans* var. *homari*.

Except for gaffkaemia in lobsters, other bacterial infections of shellfish are caused by facultative pathogens, which become invasive and destructive when host defenses are breached by injury or lowered by abnormal environmental conditions or when infection pressure is increased by population expansion of the pathogens. The vibrios and their relatives are best known for their opportunistic behavior, and vibrio infections have been reported frequently in molluscs and crustaceans.

V. ADDENDUM: CHLAMYDIAL AND RICKETTSIAL INFECTIONS OF MOLLUSCS AND CRUSTACEANS

As an addendum to this chapter, some mention should be made of chlamydial and rickettsial infections of molluscs and crustaceans, since several recent papers suggest a possibly greater role for these groups of microorganisms than has been recognized. Of particular interest is a report of systemic chlamydial infections of Dungeness crabs, *Cancer*

magister, from the Pacific coast of the United States (Sparks *et al.*, 1985). The disease may have been implicated in persistent winter mortalities of crabs in Willapa Bay and northern Puget Sound. Chlamydia-like organisms have also been seen in hard clams, *Mercenaria mercenaria*, from Chesapeake Bay and Great South Bay, New York (Harshbarger *et al.*, 1977; Meyers, 1979; Page and Cutlip, 1982), but none of the molluscan chlamydia has been associated with severe disease thus far.

Rickettsiales-like infections have been reported in shore crabs, *Carcinus mediterraneus*, from France. The organisms were transmitted to healthy crabs by inoculation and caused death in 2 weeks (Bonami and Pappalardo, 1980). Rickettsial infections were also reported in blue king crabs, *Paralithodes platypus*, from Alaska (Johnson, 1984). Additionally, penaeid shrimp, *Penaeus marginatus*, from Hawaii were found to have heavy infections of hepatopancreatic cells with rickettsial microcolonies (Brock *et al.*, 1986). The pathogens were highly infectious for juvenile *Penaeus stylirostris* in experimental studies, causing mortalities greater than 85%.

Among the molluscs, a rickettsiales-like organism infects the gills of sea scallops, *Placopecten magellanicus*, in New England waters, and has been thought to contribute indirectly to mortalities by reducing host mobility (Gulka *et al.*, 1983; Gulka and Chang, 1984). Similarly, gill infections with rickettsiales-like organisms were thought to contribute to mass mortalities of scallops, *Pecten maximus*, on the Brittany coast of France (LeGall *et al.*, 1988). Gill infections of Japanese scallops, *Patinopecten yessoensis*, on the other hand, seemed to have little deleterious effect on the hosts (Elston, 1986). Rickettsias were also found in bay scallops, *Argopecten irradians*, and mussels, *Mytilus edulis*, from New England (Morrison and Shum, 1983; Gulka and Chang, 1984). The prevalence of infections in mussels reached 40% in one population. Still other rickettsiales-like organisms have been found in oysters, *Ostrea edulis*, *Crassostrea angulata*, and *C. gigas*, from France (Comps *et al.*, 1977, 1979; Comps and Deltreil, 1979; Comps, 1980); in soft clams, *Mya arenaria*, from Chesapeake Bay (Harshbarger *et al.*, 1977; Otto *et al.*, 1979); in razor clams, *Siliqua patula*, from the Pacific Northwest (Elston and Peacock, 1989); and in the marine bivalve, *Tellina tenuis*, from Scotland (Buchanan, 1978, 1979). Infections of *Tellina* caused some pathology, particularly necrosis of digestive gland cells and abnormally thin shells.

It seems from these reports of recent vintage that chlamydia-like and rickettsiales-like microorganisms are not uncommon in marine Crustacea and Mollusca, but a strong pathogenic role has not yet been clearly demonstrated. Similarly, any links with comparable organisms in humans are largely conjectural, based on general morphology—al-

though some limited biochemical/serological evidence for a relationship has been offered (Meyers, 1979; Page and Cutlip, 1982). [It should be noted here that chlamydia-like gill infections have also been reported in several species of marine fish. Known as "epitheliocystis," the infections are usually infrequent, and reported pathogenicity has been slight.]

REFERENCES

- Anderson, J. I. W., and Conroy, D. A. (1968). The significance of disease in preliminary attempts to raise Crustacea in sea water. *Bull. Off. Int. Epizoot.* **69**, 1239–1247.
- Arie, B., and Stewart, J. E. (1984). Further developments in the induction of resistance in *Homarus americanus* to the bacterial disease gaffkemia. *Int. Counc. Explor. Sea, Doc. C.M.1984/F:9*, 3 pp.
- Baross, J. A., and Tester, P. A. (1975). Incidence and etiology of exoskeleton erosion in the spider crab, *Chionoecetes tanneri* Rathbun (Brachyura: Majidae). *Abstr. Soc. Invertebr. Pathol.* 3 pp.
- Baross, J. A., Tester, P. A., and Morita, R. Y. (1978). Incidence, microscopy, and etiology of exoskeleton lesions in the tanner crab, *Chionoecetes tanneri*. *J. Fish. Res. Board Can.* **35**, 1141–1149.
- Benton, A. G. (1935). Chitinivorous bacteria. A preliminary survey. *J. Bacteriol.* **29**, 449–463.
- Bonami, J.-R. and Pappalardo, R. (1980). Rickettsial infection in marine Crustacea. *Experimentia* **36**, 180–181.
- Bowser, P. R., Rosemark, R., and Reiner, C. R. (1981). A preliminary report of vibriosis in cultured American lobsters, *Homarus americanus*. *J. Invertebr. Pathol.* **37**, 80–85.
- Brinkley, A. W., Rommel, F. A., and Huber, T. W. (1976). The isolation of *Vibrio parahaemolyticus* and related vibrios from moribund aquarium lobsters. *Can. J. Microbiol.* **22**, 315–317.
- Brock, J. A., Nakagawa, L. K., Hayashi, T., Teruya, S. and Van Campen, H. (1986). Hepatopancreatic rickettsial infection of the penaeid shrimp, *Penaeus marginatus* (Randall), from Hawaii. *J. Fish Dis.* **9**, 73–77.
- Brown, C. (1973). The effects of some selected bacteria on embryos and larvae of the American oyster, *Crassostrea virginica*. *J. Invertebr. Pathol.* **21**, 215–223.
- Brown, C. (1981). A study of two shellfish-pathogenic *Vibrio* strains isolated from a Long Island hatchery during a recent outbreak of disease. *J. Shellfish. Res.* **1**, 83–87.
- Brown, C., and Losee, E. (1978). Observations on natural and induced epizootics of vibriosis in *Crassostrea virginica* larvae. *J. Invertebr. Pathol.* **31**, 41–47.
- Buchanan, J. S. (1978). Cytological studies on a new species of rickettsia found in association with a phage in the digestive gland of the marine bivalve mollusc, *Tellina tenuis* (da Costa). *J. Fish Dis.* **1**, 27–43.
- Buchanan, J. S. (1979). Ultrastructural studies of a rickettsialike organism (with phage) from the digestive gland of the marine bivalve, *Tellina tenuis* (da Costa). *Haliotis* **8**, 309–316.
- Castagna, M. (1975). Culture of the bay scallop, *Argopecten irradians*, in Virginia. *Mar. Fish. Rev.* **37**(1), 19–24.
- Cipriani, G. R., Wheeler, R. S., and Sizemore, R. K. (1980). Characterization of brown spot disease of Gulf coast shrimp. *J. Invertebr. Pathol.* **36**, 255–263.

- Colwell, R. R., and Sparks, A. K. (1967). Properties of *Pseudomonas enalia*, a marine bacterium pathogenic for the invertebrate *Crassostrea gigas* (Thunberg). *Appl. Microbiol.* **15**, 980–986.
- Colwell, R. R., Wicks, T. C., and Tubiash, H. S. (1975). A comparative study of the bacterial flora of the hemolymph of *Callinectes sapidus*. *Mar. Fish. Rev.* **37**(5–6), 29–33.
- Comps, M. (1980). Infections rickettsiennes chez les mollusques bivalves marins. *Haliotis* **10**(2), 39.
- Comps, M., and Deltreil, J.-P. (1979). Un microorganisme de type rickettsien chez l'huître portugaise *Crassostrea angulata* Lmk. *C. R. Hebd. Séances Acad. Sci., Ser. D* **289**, 169–171.
- Comps, M., Bonami, J.-R., and Vago, C. (1977). Pathologie des invertébrés—Mise en évidence d'une infection rickettsienne chez les huîtres. *C. R. Hebd. Séances Acad. Sci., Ser. D* **285**, 427–429.
- Comps, M., Razet, D., and Cousserans, F. (1979). Infection chlamydienne chez le Avignon *Scrobicularia piperata* (P.) du Bassin de Marennes-Oleron. *Rev. Trav. Inst. Pêch. Marit*, **43**, 413–415.
- Cook, D. W., and Lofton, S. R. (1973). Chitinoclastic bacteria associated with shell disease in *Penaeus shrimp* and the blue crab. *J. Wildl. Dis.* **9**, 154–159.
- Cornick, J. W., and Stewart, J. E. (1966). Microorganisms isolated from the hemolymph of the lobster (*Homarus americanus*). *J. Fish. Res. Board Can.* **23**, 1451–1454.
- Cornick, J. W., and Stewart, J. E. (1968a). Interaction of the pathogen *Gaffkya homari* with natural defense mechanisms of *Homarus americanus*. *J. Fish. Res. Board Can.* **25**, 695–709.
- Cornick, J. W., and Stewart, J. E. (1968b). Pathogenicity of *Gaffkya homari* for the crab, *Cancer irroratus*. *J. Fish. Res. Board Can.* **25**, 795–799.
- Cornick, J. W., and Stewart, J. E. (1975). Red crab (*Geryon quinquedens*) and snow crab (*Chionoecetes opilio*) resistance to infection by the lobster pathogen *Aerococcus viridans* (var.) *homari*. *J. Fish. Res. Board Can.* **32**, 175–180.
- Deibel, R. H., and Niven, C. F., Jr. (1960). Comparative study of *Gaffkya homari*, *Aerococcus viridans*, tetrad-forming cocci from meat-curing brines, and the genus *Pediococcus*. *J. Bacteriol.* **79**, 175–180.
- DiSalvo, L. H., Blecka, J., and Zebal, R. (1978). *Vibrio anguillarum* and larval mortality in a California coastal shellfish hatchery. *Appl. Environ. Microbiol.* **35**, 219–221.
- Editorial Secretary (1971). Rejection of the generic name *Gaffkya* Trevisan. *Int. J. Syst. Bacteriol.* **29**, 104–105.
- Elston, R. A. (1986). Occurrence of branchial rickettsiales-like infections in two bivalve molluscs, *Tapes japonica* and *Patinopecten yessoensis*, with comments on their significance. *J. Fish Dis.* **9**, 69–71.
- Elston, R., and Leibovitz, L. (1980). Detection of vibriosis in hatchery reared larval oysters: Correlation between clinical, histological and ultrastructural observations in experimentally induced disease. *Proc. Natl. Shellfish. Assoc.* **70**, 122–123.
- Elston, R., and Lockwood, G. S. (1983). Pathogenesis of vibriosis in cultured juvenile red abalone, *Haliotis rufescens* Swainson. *J. Fish Dis.* **6**, 111–128.
- Elston, R. A., and Peacock, M. G. (1989). A rickettsiales-like infection in the Pacific razor clam, *Siliqua patula*. *J. Invertebr. Pathol.* **44**, 84–96.
- Engel, D. W., and Noga, E. J. (1989). Shell disease in the blue crabs of the Pamlico River. *Environ. February*, 3–5.
- Fisher, W. S. (1976). Relationships of epibiotic fouling and mortalities of the eggs of the Dungeness crab (*Cancer magister*). *J. Fish. Res. Board Can.* **38**, 2849–2853.
- Fisher, W. S. (1977). Shell disease of lobsters. In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann, ed.), pp. 158–162. Elsevier, Amsterdam.

- Fisher, W. S., Rosemark, T. R., and Nilson, E. H. (1976). The susceptibility of cultured American lobsters to a chitinolytic bacterium. *Proc. Annu. Meet.—World Maric. Soc.* **7**, 511–520.
- Fisher, W. S., Nilson, E. H., Steenbergen, J. F., and Lightner, D. V. (1978). Microbial diseases of cultured lobsters: A review. *Aquaculture* **14**, 115–140.
- Fisher, W. S., Rittenburg, J. H., Bayer, R. C., and Leavitt, D. F. (1979). Incidence of *Aerococcus viridans* (var.) *homari* in natural crab (*Cancer irroratus*, *Cancer borealis*) populations from Maine coastal waters. *Crustaceana* **37**, 316–317.
- Fujita, T., Matsubara, T., Hirokawa, H., and Araki, F. (1953). On the inflammatorious changes of the *Ostrea gigas* in Hiroshima Bay. *Nippon Suisan Gakkaishi* **19**, 766–770 (in Japanese; English summary).
- Fujita, T., Matsubara, T., Hirokawa, H., and Araki, F. (1955). On the inflammatorious changes of the *Ostrea gigas* in Hiroshima Bay. II. *Nippon Suisan Gakkaishi* **20**, 1063–1065 (in Japanese; English summary).
- Getchell, J. S. (1949). A study of abnormal shrinkage of Maine lobsters ("red tail") with observations and recommendations. *Maine Dep. Sea Shore Fish. Res. Bull.* 6 pp.
- Gibson, F. A. (1961). *Gaffkaemia* in stored lobsters. *Cons. Perm. Explor. Mer, Shellfish Comm. Doc.* **58**, 1 p. (mimeo).
- Goggins, P. L., and Hurst, J. W., Jr. (1960). Progress report on lobster gaffkyaremia (red tail). *Maine Dep. Sea Shore Fish. Bull.* 9 pp. (unpublished mimeo).
- Gordon, I. (1966). Parasites and diseases of Crustacea. *Mem. Inst. Found. Afr. Noire* **77**, 27–86.
- Grishchowsky, R. S., and Liston, J. (1974). Bacterial pathogenicity in laboratory-induced mortality of the Pacific oyster (*Crassostrea gigas* Thunberg). *Proc. Natl. Shellfish. Assoc.* **64**, 82–91.
- Guillard, R. R. L. (1959). Further evidence of the destruction of bivalve larvae by bacteria. *Biol. Bull. (Woods Hole, Mass.)* **117**, 258–266.
- Gulka, G., and Chang, P. W. (1984). Pathogenicity and infectivity of a rickettsia-like organism in the sea scallop, *Placopecten magellanicus*. *J. Fish Dis.* **8**, 309–318.
- Gulka, G., Chang, P. W., and Marti, K. A. (1983). Prokaryotic infection associated with a mass mortality of the sea scallop, *Placopecten magellanicus*. *J. Fish Dis.* **6**, 355–364.
- Hanlon, R. T., Forsythe, J. W., Cooper, K. M., Dinuzzo, A. R., Folse, D. S., and Kelly, M. T. (1984). Fatal penetrating skin ulcers in laboratory-reared octopuses. *J. Invertebr. Pathol.* **44**, 67–83.
- Harshbarger, J. C., Chang, S. C., and Otto, S. V. (1977). Chlamydiae (with phages), mycoplasmas, and Rickettsiae in Chesapeake Bay bivalves. *Science* **196**, 666–668.
- Helm, M. M., and Smith, F. M. (1971). Observations on a bacterial disease in laboratory-cultured larvae of the European flat oyster, *Ostrea edulis* L. *Int. Counc. Explor. Sea., Doc. C.M.1971/K:10*, 7 pp.
- Hess, E. (1937). A shell disease in lobsters (*Homarus americanus*) caused by chitinovorous bacteria. *J. Biol. Board Can.* **3**, 358–362.
- Hitchner, E. R., and Snieszko, S. F. (1947). A study of a microorganism causing a bacterial disease of lobster. *J. Bacteriol.* **54**, 48 (abstract).
- Hock, C. W. (1940). Decomposition of chitin by marine bacteria. *Biol. Bull. (Woods Hole, Mass.)* **79**, 199–206.
- Hock, C. W. (1941). Marine chitin-decomposing bacteria. *J. Mar. Res.* **4**, 99–106.
- Hood, M. A., and Meyers, S. P. (1974). Distribution of chitinoclastic bacteria in natural estuarine waters and aquarial systems. *Proc. Gulf Coast Reg. Symp. Dis. Aquat. Anim.* **LSU-SG-74-05**, 115–121.

- Hulet, W. H., Villoch, M. R., Hixon, R. F., and Hanlon, R. T. (1979). Fin damage in captured and reared squids. *Lab. Anim. Sci.* **29**, 528–533.
- Iversen, E. S., and Beardsley, G. L. (1976). Shell disease in crustaceans indigenous to South Florida. *Prog. Fish-Cult.* **38**, 195–196.
- Jeffries, V. E. (1982). Three *Vibrio* strains pathogenic to larvae of *Crassostrea gigas* and *Ostrea edulis*. *Aquaculture* **29**, 201–226.
- Johnson, P. T. (1976). Bacterial infection in the blue crab, *Callinectes sapidus*: Course of infection and histopathology. *J. Invertebr. Pathol.* **28**, 25–36.
- Johnson, P. T. (1984). A rickettsia of the blue king crab, *Paralithodes platypus*. *J. Invertebr. Pathol.* **44**, 112–113.
- Johnson, P. T., Stewart, J. E., and Arie, B. (1981). Histopathology of *Aerococcus viridans* (var.) *homari* infection (gaffkemia) in the lobster, *Homarus americanus*, and a comparison with histological reactions to a gram-negative species, *Pseudomonas perolens*. *J. Invertebr. Pathol.* **38**, 127–148.
- Johnson, S. K. (1974). Ectocommensals and parasites of shrimp from Texas rearing ponds. *Tex. A&M Univ. Sea Grant Program [Rep.] TAMU-SG-74-207*, 20 pp.
- Kan-no, H., Sasaki, M., Sakurai, Y., Watanabe, T., and Suzuki, K. (1965). Studies on the mass mortality of the oyster in Matsushima Bay. I. General aspect of the mass mortality of the oyster in Matsushima Bay and its environmental conditions. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 1–26 (in Japanese; English summary).
- Kelly, K. F., and Evans, J. B. (1974). Deoxyribonucleic acid homology among strains of the lobster pathogen *Gaffkya homari* and *Aerococcus viridans*. *J. Gen. Microbiol.* **81**, 257–260.
- Kocur, M., and Martinec, T. (1965). Proposal for the rejection of the bacterial generic name *Gaffkya*. *Int. Bull. Bacteriol. Nomencl. Taxon.* **15**, 177–179.
- Krantz, G. E., Colwell, R. R., and Lovelace, E. (1969). *Vibrio parahaemolyticus* from the blue crab *Callinectes sapidus* in Chesapeake Bay. *Science* **164**, 1286–1287.
- Lauckner, G. (1972). "Zur Taxonomie, Ökologie und Physiologie von *Cardium edule* L. und *Cardium lamarcki* Reeve," dissertation. University of Kiel, Kiel, FRG.
- Lear, D. W., Jr. (1963). Occurrence and significance of chitinoclastic bacteria in pelagic waters and zooplankton. In "Symposium on Marine Microbiology" (C. Oppenheimer, ed.), pp. 594–610. Thomas, Springfield, Illinois.
- Legall, G., Chagot, D., Mialhe, E., and Grizel, H. (1988). Branchial rickettsiales-like infection associated with a mass mortality of sea scallop *Pecten maximus*. *Dis. Aquat. Org.* **4**, 229–232.
- Leibovitz, L. (1978a). Bacteriologic studies of Long Island shellfish hatcheries: An abstract. *Mar. Fish. Rev.* **40**(10), 8.
- Leibovitz, L. (1978b). Shellfish diseases. *Mar. Fish. Rev.* **40**(3), 61–64.
- Leibovitz, L. (1978c). A study of vibriosis at a Long Island shellfish hatchery. *Int. Council. Explor. Sea, Doc.* **C.M.1978/F:17**, 23 pp.
- Leibovitz, L. (1979). A study of vibriosis at a Long Island shellfish hatchery. *Proc. Natl. Shellfish. Assoc.* **69**, 169.
- Leibovitz, L., and Elston, R. (1980). Detection of vibriosis in hatchery larval oyster cultures: Study of the interrelationship of diagnosis and management variables in an experimental model. *Proc. Natl. Shellfish. Assoc.* **70**, 123–125.
- Leibovitz, L., Meyers, T. R., Elston, R., and Chanley, P. (1977). Necrotic exfoliative dermatitis of captive squid (*Loligo pealei*). *J. Invertebr. Pathol.* **30**, 369–376.
- Lewis, D. H. (1973). Response of brown shrimp to infection with *Vibrio* sp. *Proc. Annu. Meet.—World Maric. Soc.* **4**, 333–338.
- Lightner, D. V. (1977a). *Vibrio* disease of shrimps. In "Disease Diagnosis and Control in

- North American Marine Aquaculture" (C. J. Sindermann, ed.), pp. 19–26. Elsevier, Amsterdam.
- Lightner, D. V. (1977b). Filamentous bacterial disease of shrimps. In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann, ed.), pp. 31–35. Elsevier, Amsterdam.
- Lightner, D. V. (1983). Diseases of cultured penaeid shrimp. In "CRC Handbook of Mariculture. Crustacean Aquaculture" (J. P. McVey, ed.), pp. 289–320. CRC Press, Boca Raton, Florida.
- Lightner, D. V., Redman, R. M., Danald, D. A., Williams, R. R., and Perez, L. A. (1984). Major diseases encountered in controlled environment culture of penaeid shrimp at Puerto Peñasco, Soñora, Mexico. In "Proceedings of the Ninth and Tenth U.S.–Japan Meetings on Aquaculture" (C. J. Sindermann, ed.), NOAA Tech. Rep. NMFA 16, pp. 25–33. U.S. Dep. Commerce, Washington, D.C.
- Lipovsky, V. P. and Chew, K. K. (1971). A preliminary report on Pacific oyster (*Crassostrea gigas*) mortality after transfer from a natural bed into 10°C and 20°C water in the laboratory. *Proc. Natl. Shellfish. Assoc.* **61**, 9.
- Lipovsky, V. P., and Chew, K. K. (1972). Mortality of Pacific oysters (*Crassostrea gigas*): The influence of temperature and enriched seawater on oyster survival. *Proc. Natl. Shellfish. Assoc.* **62**, 72–82.
- Lipovsky, V. P., and Chew, K. K. (1973). Laboratory control of Pacific oyster mortality by manipulation of temperature and nutrient concentration. *Proc. Natl. Shellfish. Assoc.* **63**, 3.
- Lipp, P. R., Brown, B., Liston, J., and Chew, K. K. (1976). Recent findings on the summer disease of Pacific oysters. *Proc. Natl. Shellfish. Assoc.* **65**, 9–10.
- Loosanoff, V. L. (1974). Factors responsible for the mass mortalities of molluscan larvae in nature: A review. *Proc. Ann. Meet.—World Maric. Soc.* **5**, 297–309.
- Malloy, S. C. (1978). Bacteria induced shell disease of lobsters (*Homarus americanus*). *J. Wildl. Dis.* **14**, 2–10.
- Mann, H. (1939). Die Brandfleckenkrankheit beim Sumpfkrebs (*Potamobius leptodactylus* Eschh.). *Z. Parasitenkd.* **11**, 430–432.
- Mann, H., and Pieplow, U. (1938). Die Brandfleckenkrankheit bei Krebsen und ihre Erreger. *Z. Fisch.* **36**, 225–240.
- Martin, Y. (1976). Importance des bactéries chez les mollusques bivalves. *Haliotis* **7**, 97–103.
- McLeese, D. W. (1965). Lesions on the abdominal membrane of lobsters. *J. Fish. Res. Board Can.* **11**, 639–640.
- Meyers, T. R. (1979). Preliminary studies on a chlamydial agent in the digestive diverticular epithelium of hard clams *Mercenaria mercenaria* (L.) from Great South Bay, New York. *J. Fish Dis.* **1**, 179–189.
- Mori, K., Imai, T., Toyoshima, K., and Usuki, I. (1965a). Studies on the mass mortality of the oyster in Matsushima Bay. IV. Changes in the physiological activity and the glycogen content of the oyster during the stages of sexual maturation and spawning. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 49–63 (in Japanese; English summary).
- Mori, K., Tamate, H., Imai, T., and Itikawa, O. (1965b). Studies on the mass mortality of the oyster in Matsushima Bay. V. Changes in the metabolism of lipids and glycogen of the oyster during the stages of sexual maturation and spawning. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 65–88 (in Japanese; English summary).
- Morrison, C., and Shum, G. (1983). Chlamydia-like organisms in the digestive diverticula of the bay scallop, *Argopecten irradians* (Lmk.). *J. Fish Dis.* **5**, 173–184.
- Murchelano, R. A., and Bishop, J. L. (1969). Bacteriological study of laboratory-reared juvenile American oysters *Crassostrea virginica*. *J. Invertebr. Pathol.* **14**, 321–327.

- Murchelano, R. A., and Brown, C. (1968). Bacteriological study of the natural flora of the eastern oyster, *Crassostrea virginica*. *J. Invertebr. Pathol.* **11**, 519–520.
- Murchelano, R. A., and Brown, C. (1969). Bacterial flora of some algal foods used for rearing bivalve larvae. *J. Fish. Res. Board Can.* **26**, 2760–2764.
- Newman, M. C., and Feng, S. Y. (1982). Susceptibility and resistance of the rock crab, *Cancer irroratus*, to natural and experimental bacterial infection. *J. Invertebr. Pathol.* **40**, 75–88.
- Nilson, E. H., Fisher, W. S., and Shleser, R. A. (1975). Filamentous infestations observed on eggs and larvae of cultured crustaceans. *Proc. Annu. Meet.—World Mari. Soc.* **6**, 367–375.
- Numachi, K., Oizumi, J., Sato, S., and Imai, T. (1965). Studies on the mass mortality of the oyster in Matsushima Bay. III. The pathological changes of the oyster caused by gram-positive bacteria and the frequency of their infection. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 39–48 (in Japanese; English summary).
- Otto, S. V., Harshbarger, J. C., and Chang, S. C. (1979). Status of selected unicellular eucaryote pathogens, and prevalence and histopathology of inclusions containing obligate procaryote parasites, in commerical bivalve mollusks from Maryland estuaries. *Haliotis* **8**, 285–295.
- Overstreet, R. M. (1978). Marine Maladies? Worms, Germs, and Other Symbionts from the Northern Gulf of Mexico. *Miss.-Ala. Sea Grant Consortium, Publ. MASGP-78-021*, 140 pp.
- Page, L. A., and Cutlip, R. C. (1982). Morphological and immunological confirmation of the presence of Chlamydiae in the gut tissues of the Chesapeake Bay clam, *Mercenaria mercenaria*. *Curr. Microbiol.* **7**, 297–300.
- Quayle, D. B. (1969). Pacific oyster culture in British Columbia. *Bull. Fish. Res. Board Can.* **169**, 1–192.
- Rabin, H. (1965). Studies on gaffkemia, a bacterial disease of the American lobster, *Homarus americanus* (Milne-Edwards). *J. Invertebr. Pathol.* **7**, 391–397.
- Rabin, H., and Hughes, J. T. (1968). Studies on host–parasite relationships in gaffkemia. *J. Invertebr. Pathol.* **10**, 335–344.
- Regal, P. J. (1986). Models of genetically engineered organisms and their ecological impact. In “Ecology of Biological Invasions of North America and Hawaii” (H. A. Mooney and J. A. Drake, eds.), pp. 111–129. Springer-Verlag, New York.
- Roald, S. O., Aursjø, J., and Håstein, T. (1981). Occurrence of shell disease in lobsters, *Homarus gammarus* (L.), in the southern part of the Oslofjord, Norway. *Fisk. Dir. Skr. Ser. Havunders.* **17**, 153–160.
- Rosen, B. (1967). Shell disease of the blue crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* **9**, 348–353.
- Rosen, B. (1970). Shell disease of aquatic crustaceans. In “A Symposium on Diseases of Fishes and Shellfishes” (S. F. Snieszko, ed.), Spec. Publ. 5, pp. 409–415. Am. Fish. Soc., Washington, D.C.
- Roskam, R. T. (1957). Gaffkaemia, a contagious disease, in *Homarus vulgaris*. *Cons. Perm. Int. Explor. Mer, Shellfish Comm. Rep.* **4** pp. (mimeo).
- Sandifer, P. A., and Eldridge, P. J. (1974). Observations on the incidence of shell disease in South Carolina blue crabs, *Callinectes sapidus*. *Proc. Gulf Coast Reg. Symp. Dis. Aquat. Anim.* **LSU-SG-74-05**, 161–184.
- Sawyer, W. H., Jr, and Taylor, C. C. (1949). The effect of shell disease on the gills and chitin of the lobster (*Homarus americanus*). *Maine Dep. Sea Shore Fish., Res. Bull.* **1**, 1–10.
- Schäfer, W. (1954). Form und Funktion der Brachyuren-Schere. *Abh. Senckenb. Naturforsch. Ges.* **489**, 1–65.

- Sindermann, C. J., and Rosenfield, A. (1967). Principal diseases of commercially important marine bivalve Mollusca and Crustacea. *Fish. Bull.* **66**, 335–385.
- Snieszko, S. F., and Taylor, C. C. (1947). A bacterial disease of the lobster (*Homarus americanus*). *Science* **105**, 500.
- Sparks, A. K. (1981). Bacterial diseases of invertebrates other than insects. In "Pathogenesis of Invertebrate Microbial Diseases" (E. W. Davidson, ed.), pp. 323–363. Allanheld & Osmun, Totowa, New Jersey.
- Sparks, A. K., Morado, J. F., and Hawkes, J. W. (1985). A systemic microbial disease in the Dungeness crab, *Cancer magister*, caused by a Chlamydia-like organism. *J. Invertebr. Pathol.* **45**, 204–217.
- Spindler-Barth, M. (1976). A bacterial infection in the common shore crab *Carcinus maenas* and the fiddler crab *Uca pugilator*. *Mar. Biol. (Berlin)* **36**, 1–4.
- Stewart, J. E. (1975). Gaffkemia, the fatal infection of lobsters (genus *Homarus*) by *Aerococcus viridans* (var.) *homari*: A review. *Mar. Fish. Rev.* **37**(5–6), 20–24.
- Stewart, J. E. (1978). The lobster disease, gaffkemia, in relation to fisheries management. *Int. Council. Explor. Sea, Shellfish Comm. Doc. C. M.* **1978/F:16**, 1–8.
- Stewart, J. E. (1980). Diseases. In "The Biology and Management of Lobsters" (J. S. Cobb and B. F. Philipps, eds), Vol. I, pp. 301–342. Academic Press, New York.
- Stewart, J. E. (1984). Lobster diseases. *Helgol. Wiss. Meeresunters.* **37**, 243–254.
- Stewart, J. E., and Arie, B. (1973a). Paradoxical effects of salinity reductions on lobsters (*Homarus americanus*) infected with *Gaffkya homari*. *Comp. Biochem. Physiol. A* **45**, 717–730.
- Stewart, J. E., and Arie, B. (1973b). Depletion of glycogen and adenosine triphosphate as major factors in the death of lobsters (*Homarus americanus*) infected with *Gaffkya homari*. *Can. J. Microbiol.* **19**, 1103–1110.
- Stewart, J. E., and Arie, B. (1974). Effectiveness of vancomycin against gaffkemia, the bacterial disease of lobsters (genus *Homarus*). *J. Fish. Res. Board Can.* **31**, 1873–1879.
- Stewart, J. E., and Cornick, J. W. (1967). In vitro susceptibilities of the lobster pathogen *Gaffkya homari* to various disinfectants and antibiotics. *J. Fish. Res. Board Can.* **24**, 2623–2626.
- Stewart, J. E., and Cornick, J. W. (1972). Effects of *Gaffkya homari* on glucose, total carbohydrates, and lactic acid of the hemolymph of the lobster (*Homarus americanus*). *Can. J. Microbiol.* **18**, 1511–1513.
- Stewart, J. E., and Dingle, J. R. (1968). Characteristics of hemolymphs of *Cancer irroratus*, *C. borealis*, and *Hyas coactatus*. *J. Fish. Res. Board Can.* **25**, 607–610.
- Stewart, J. E., and MacDonald, J. F. (1962). A report to the fishing industry regarding lobster disease (gaffkemia). *Fish. Res. Board Canada, Technol. Stn., Halifax, New Ser. Circ.* **9**, 2 pp.
- Stewart, J. E., and Rabin, H. (1970). Gaffkemia, a bacterial disease of lobsters (genus *Homarus*). In "A Symposium on Diseases of Fishes and Shellfishes" (S. F. Snieszko, ed.), Spec. Publ. 5, pp. 431–439. Am. Fish. Soc., Washington, D.C.
- Stewart, J. E., and Zwicker, B. M. (1972). Natural and induced bactericidal activities in the hemolymph of the lobster, *Homarus americanus*: Products of hemocyte–plasma interaction. *Can. J. Microbiol.* **18**, 1499–1509.
- Stewart, J. E., and Zwicker, B. M. (1974). Comparison of various vaccines for inducing resistance in the lobster *Homarus americanus* to the bacterial infection, gaffkemia. *J. Fish. Res. Board Can.* **31**, 1887–1892.
- Stewart, J. E., Cornick, J. W., Spear, D. I., and McLeese, D. W. (1966). Incidence of *Gaffkya homari* in natural lobster (*Homarus americanus*) populations of the Atlantic region of Canada. *J. Fish. Res. Board Can.* **23**, 1325–1330.

- Stewart, J. E., Arie, B., Zwicker, B. M., and Dingle, J. R. (1969a). Gaffkemia, a bacterial disease of the lobster, *Homarus americanus*: Effects of the pathogen, *Gaffkya homari*, on the physiology of the host. *Can. J. Microbiol.* **15**, 925–932.
- Stewart, J. E., Cornick, J. W., and Zwicker, B. M. (1969b). Influence of temperature on gaffkemia, a bacterial disease of the lobster *Homarus americanus*. *J. Fish. Res. Board Can.* **26**, 2503–2510.
- Stewart, J. E., Dockrill, A., and Cornick, J. W. (1969c). Effectiveness of the integument and gastric fluid as barriers against transmission of *Gaffkya homari* to the lobster *Homarus americanus*. *J. Fish. Res. Board Can.* **26**, 1–14.
- Stewart, J. E., Foley, D. M., and Ackman, R. G. (1969c). Characteristics of *Gaffkya homari*, the causative agent of the lobster disease gaffkemia. *J. Fish. Res. Board Can.* **26**, 1385–1389.
- Stewart, J. E., Horner, G. W., and Arie, B. (1972a). Effects of temperature, food, and starvation on several physiological parameters of the lobster *Homarus americanus*. *J. Fish. Res. Board Can.* **29**, 439–442.
- Stewart, J. E., Zwicker, B. M., Arie, B., and Horner, G. W. (1972b). Food and starvation as factors affecting the time to death of the lobster *Homarus americanus* infected with *Gaffkya homari*. *J. Fish. Res. Board Can.* **29**, 461–464.
- Stewart, J. E., Zwicker, B. M., and Arie, B. (1975). Status report on gaffkemia in lobsters in Atlantic Canada. *Int. Counc. Explor. Sea, Doc. C.M.1975/K:20*, 4 pp.
- Takahashi, Y., Shimoyama, Y., and Momoyama, K. (1985). Pathogenicity and characteristics of *Vibrio* sp. isolated from cultured Kuruma prawn *Penaeus japonicus* Bate. *Nippon Suisan Gakkaishi* **51**, 721–730 (in Japanese; English abstract).
- Takeuchi, T., Takemoto, Y., and Matsubara, T. (1960). Haematological study of bacteria affected oysters. *Hiroshima-ken Suisan Shikenjo Jikyo Hokoku* **11**(1), 1–7 (in Japanese; translated by U.S. Joint Publ. Res. Serv. for Transl. Prog., Bur. Commer. Fish., Milford, Connecticut, 1965).
- Tamate, H., Numachi, K., Mori, K., Itikawa, O., and Imai, T. (1965). Studies on the mass mortality of the oyster in Matsushima Bay. VI. Pathological studies. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 89–104 (in Japanese; English summary).
- Taylor, C. C. (1948). Shell disease as a mortality factor in the lobster (*Homarus americanus*). *Maine Dep. Sea Shore Fish. Fish. Circ.* **4**, 1–8 (mimeo).
- Taylor, C. C. (1949). A study of lobster shell disease with observations and recommendations. *Maine Dep. Sea Shore Fish. Res. Bull.* 5 pp.
- Tubiash, H. S., and Krantz, G. E. (1970). Experimental bacterial infection of the blue crab, *Callinectes sapidus*. *Am. Soc. Microbiol. Annu. Meet. Abstr.* G80.
- Tubiash, H. S., Chanley, P. E., and Leifson, E. (1965). Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. I. Etiology and epizootiology. *J. Bacteriol.* **90**, 1036–1044.
- Tubiash, H. S., Colwell, R. R., and Sakazaki, R. (1970). Marine vibrios associated with bacillary necrosis, a disease of larval and juvenile bivalve mollusks. *J. Bacteriol.* **103**, 271–272.
- Tubiash, H. S., Otto, S. V., and Hugh, R. (1973). Cardiac edema associated with *Vibrio anguillarum* in the American oyster. *Proc. Natl. Shellfish. Assoc.* **63**, 39–42.
- Vachon, N. S., Bayer, R. C., and Rittenburg, J. H. (1981). Incidence of *Aerococcus viridans* (var.) *homari* in American lobster populations from the Gulf of Maine. *Prog. Fish-Cult.* **43**, 49.
- Vanderzant, C., Mroz, E., and Nickelson, R. (1970). Microbial flora of Gulf of Mexico and pond shrimp. *J. Milk Food Technol.* **33**, 346–350.
- Vanderzant, C., Nickelson, R. N., and Judkins, P. W. (1971). Microbial flora of pond-reared brown shrimp (*Penaeus aztecus*). *Appl. Microbiol.* **21**, 916–921.

- Wood, P. C. (1965a). A preliminary note on Gaffkaemia investigations in England. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **156**, 30–34.
- Wood, P. C. (1965b). Gaffkaemia, the blood disease of lobsters. *Proc. Soc. Gen. Microbiol.* p. 14 (abstract).
- Young, J. S., and Pearce, J. B. (1975). Shell disease in crabs and lobsters from the New York Bight. *Mar. Pollut. Bull.* **6**, 101–105.
- Zobell, C. E., and Rittenberg, R. (1938). The occurrence and characteristics of chitinoclastic bacteria in the sea. *J. Bacteriol.* **35**, 275–287.

4

Fungi

I. INTRODUCTION

Fungi can be important to marine shellfish populations in several ways. In estimated descending order of impacts, these are:

1. As occasionally severe pathogens of molluscan and crustacean larvae and postlarvae in aquaculture operations
2. As destructive parasites of developing crustacean eggs
3. As invaders of secreted structures—shell and byssus—in bivalve molluscs, and (occasionally) the chitinous exoskeleton of crustaceans
4. As pathogens of juvenile and adult crustaceans—in wild stocks as well as in aquaculture populations

Most of the fungal pathogens that have been described are, quite logically, aquatic Phycomycetes, although occasional representatives of other groups of fungi may emerge as disease agents—especially in crustacean hosts.

This chapter attempts to summarize the scattered information on fungal diseases of economically important marine shellfish, emphasizing the more recent data on fungi as pathogens of larvae and postlarvae in aquaculture ventures.

II. MOLLUSCAN FUNGAL DISEASES

Fungi are only weakly represented among the serious pathogens of molluscs, and those that seem most important in natural populations attack external parts (shells of oysters and byssus of mussels). In molluscan hatcheries fungal pathogens of larvae may occur sporadically, but effects seem minor compared with those of vibriosis and related bacterial diseases. As might be expected, there is some disagreement as to

whether the fungal organisms seen are primary pathogens or secondary invaders.

A disease of European flat oysters, *Ostrea edulis*, called *maladie du pied*, or shell disease, has been recognized for more than a century (Montauge, 1878) and is probably the most severe known disease of fungal etiology in marine bivalves, as well as the earliest scientifically recognized disease in oysters (Sprague, 1971). It has long been known in flat oysters as *maladie du pied*, or foot disease (Dollfus, 1921). Its etiological agent was first described as a bacterium (Giard, 1894), but further studies disclosed the common occurrence of a fungus. The infection was localized in the shell under the attachment of the adductor muscle, where it caused roughening and blistering of the shell and degeneration of adjacent muscle tissue. The muscle became detached as irregular cysts were formed. Major mortalities occurred in oyster beds at Arcachon, France, in 1877 (Hornell, 1910; Orton, 1937). The cause was not determined, but some evidence of foot disease was found.

Much later, mortalities occurred in European oysters in The Netherlands, beginning in 1930 (Havinga, 1931). Studies by Korringa (1947, 1948, 1951a,b, 1952) disclosed the presence of a fungal disease (presumably *maladie du pied*), characterized by the formation of green or brown pustules on the inner shell surfaces. Activity of the fungus varied directly with temperature, and the outbreak was said to be intensified by widespread use of cockle shells as spat collectors. Thin parts of oyster shells were perforated by the disease agent, which proliferated after reaching the interior surfaces. The fungal pathogen was identified as a species of *Monilia* by Voisin (1931), who found the infection, labeled "shell disease," in 40% of oysters imported into France from The Netherlands in 1931. Cole (1950) and Cole and Waugh (1956) reported infections in the European oyster, *O. edulis*, from Brittany, and in Portuguese oysters, *Crassostrea angulata*, grown in England. Infections were common in beds where old shells were abundant. Cole and Hancock (1956) found the disease in almost all beds of native European oysters in England, and described two distinct forms: the typical one characterized by greenish, rubbery warts and knobs on the inside of the shell, particularly in the region of the muscle attachment, and an atypical form in which young oysters had thickened shell margins with numerous white patches but had no deformation of the muscle attachment area.

A definitive study of shell disease, or *maladie du pied*, was reported by Alderman and Jones (1967, 1971a,b). They concluded from observations of the organism in culture that the etiological agent, present in the shells of diseased oysters, was a phycomycetous fungus, possibly a member of the Saprolegniaceae. The shell disease in these reports occurred in native *O. edulis* in England and Ireland, but specimens re-

ceived from France were similar in gross pathological effects produced and in the vegetative structures of the fungus present. The authors suggested that the epizootic nature of the disease could be explained by massive production of zoospores by the fungus under suitable environmental conditions. They also concluded that the progression of the disease included:

1. Initial appearance of tiny, elevated chalk-white spots on the inner surface of shells, followed by coalescence of the spots to cover one or both valves.
2. Advanced infections recognizable by the development of green or brown, rubberlike knobs or warts of conchiolin on the inner surface of the shell, particularly in the region of the adductor muscle attachment.

Alderman and Jones (1971a) identified the pathogen as *Ostracoblabe implexa*, which had been described much earlier by Bornet and Flahault (1889) and had been associated with *maladie de la coquille* by Dollfus (1923). The remarkable similarity in gross signs of the disease, as reported from France, The Netherlands, and the British Isles for more than a century, suggests a specific disease entity of fungal etiology, regardless of the variability in the descriptive terms applied.

Shell disease has been reported only rarely from other geographic areas. Durve and Bal (1960) reported the occurrence of a shell disease which they considered to be *maladie du pied* in the backwater oyster, *Crassostrea gryphoides* from India, and Galtsoff (1964, 1969) reported the rare occurrence of the disease in the American oyster, *Crassostrea virginica*, from the southern United States, but did not consider it a serious threat to oyster populations. Recently, a fungus described as *O. implexa* was isolated from shell lesions of the rock oyster, *Saccostrea* (*Crassostrea*) *cucullata* in India (Raghukumar and Lande, 1988). Direct shell-to-shell transmission was achieved experimentally, but sporulation in culture was not seen. Despite these observations, shell disease seems to be a severe problem only on the northern European coast and only in *O. edulis* and *C. angulata* (Alderman and Jones, 1971a).

Another external site of fungal invasion is the byssus of certain bivalves. While fungi participate in normal degrading processes in the marine environment, the more exuberant and aggressive members of the group may on occasion have deleterious effects on living animals. A suggestion of this is contained in a report by Vitellaro-Zuccarello (1973) of the general occurrence of a fungus (possibly an ascomycete) in the byssus of *Mytilus galloprovincialis* sampled on the Italian coast. Acting saprophytically, the fungus could weaken the byssus, leading to dis-

lodgement of the mussel. The ubiquitous occurrence of the organism within the byssus, as reported by Vitellaro-Zuccarello, suggests an environmental factor of possible significance to survival.

Circumstantial evidence linking byssal degradation with mussel mortalities was reported by Lauckner (1983). Referring to recurrent mortalities in northern European waters beginning in 1901 (Hirsch, 1921; Dollfus, 1923), he pointed out that the initial and characteristic sign of the disease was degradation of byssus threads, followed by weakening of the shell ligament. Presence of a fungus was not reported, but the speculation is intriguing.

Davis *et al.* (1954) isolated a fungus, later described as a phycomycete, *Sirolpidum zoophthorum* (Vishniac, 1955), from hatchery-produced oyster and clam larvae. The infections were considered enzootic in many of the cultures, but produced occasional epizootics that killed most of the cultured larval populations in 2–4 days (Loosanoff and Davis, 1963). Juvenile as well as larval bivalves were infected, growth ceased, and death followed soon after infection. Infected cultures of bivalve larvae contained large numbers of motile, biflagellate, infective zoospores of the fungus. Loosanoff (1954, 1974) speculated that an epizootic of the fungus could occur among lamellibranch larvae in nature, and the organism has been reported from feral bivalve larvae (Johnson and Sparrow, 1961), but not at epizootic levels.

A species of *Haliphthoros*, also a phycomycete, has been proposed as a possible biological control measure for the oyster drill, *Urosalpinx cinerea*. Ganaros (1957) observed a fungus parasitic on ova within egg cases in laboratory holding tanks. Identified as *Haliphthoros milfordensis* by Vishniac (1958), the fungus was found to be infective experimentally to several stages of living *Urosalpinx* larvae but not to later developmental stages (protoconchs). Ganaros seemed unnecessarily pessimistic about the possible use of the fungus for biological control of drills, on the basis of doubtful practicability of creating environmental conditions conducive to infection and dissemination. He did believe, however, that if the snails themselves could spread the pathogen, it might prove to be an effective control method. A very interesting facet of the study was that an isolate of *Haliphthoros* sent to England was able to infect eggs of the oyster pea crab, *Pinnotheres*; in this way it resembled a fungal pathogen of crustacean eggs, *Atkinsiella (Plectospira) dubia*, first described by Atkins (1954b).

It seems relevant to this discussion that *H. milfordensis* was identified recently as the cause of an epizootic in abalone, *Haliotis sieboldii*, in a storage facility in Japan (Hatai, 1982). The disease, presumably among market-size abalones, was characterized by flat or tubercle-like swellings on mantle and foot, with fungal hyphae permeating the lesions.

Another phycomycete, *Leptolegnia marina*, normally a parasite of the pea crab, *Pinnotheres pisum*, was reported to infect cockles, *Cardium echinatum*, sampled in British waters (Atkins, 1954a). Disease signs included formation of pustules on the foot and mantle margin, and tissue invasion by fungal hyphae.

In addition to fungi with reasonably acceptable affinities, two protistan groups—the thraustochytrids and the labyrinthulids—with uncertain affinities, have been variously claimed and disowned by mycologists and protozoologists. Some investigators consider them to be lower fungi (Gaertner, 1972; Perkins, 1976a); some consider them to be Protozoa of the phylum Labyrinthomorpha (Olive, 1975; Levine *et al.*, 1980; Lauckner, 1983; Pokorny, 1985); others place them in two orders, Thraustochytriales and Labyrinthulales, with higher taxonomic affinities unspecified (Alderman *et al.*, 1974; Moss, 1985). This taxonomic confusion is unfortunate, because it detracts from the reality that certain of the thraustochytrids, thought until recently to be saprophytes, are slowly emerging as molluscan parasites of some significance (Alderman, 1986). In an early study a thraustochytridaceous fungus, *Thraustochytrium inglei*, was believed to be responsible for summer mortalities of oysters, *C. virginica*, in Florida waters (Quick, 1972a,b, 1974a,b). Cephalopods may also be infected by thraustochytrids; progressive external lesions characterized infections of the lesser octopus, *Eledone cirrhosa*, from the North Sea, and of the short-finned squid, *Illex illecebrosus*, from Canadian waters (Polglase, 1980; Jones and O'Dor, 1983). In squid the extent of the lesions in the gills led to the conclusion that the disease was the principal cause of delayed mortality in captive populations, even though no experimental infections were reported. Stresses associated with captivity were considered important in increasing susceptibility to the facultative pathogens. Juvenile abalone, *Haliotis kamtschatkana* and *H. rufescens*, were reported recently to have been killed by infections with a thraustochytrid, *Labyrinthuloides haliotidis*. Nerve and muscle tissue were invaded, the disease rapidly overwhelmed the cultured populations, and mortalities approached 100% (Bower, 1987a–d). These reports, plus a recent description of yellow spot disease of the nudibranch *Tritonia diomedea*—a disease thought to be caused by a thraustochytrid of the genus *Schizochytrium*—suggest a possibly larger role for this group of presumptive fungi as pathogens of marine molluscs (McLean and Porter, 1982; Alderman, 1986).

The assemblage of fungal pathogens of molluscs considered in this section is distressingly small. Fortunately, it may be augmented in the future by reassignment of a number of poorly defined protistans now residing uneasily in the protozoan phylum Labyrinthomorpha—after more is learned about their characteristics. This sequence of events, in

reverse, has already been the fate of one significant oyster pathogen, *Perkinsus marinus*. Described originally as a fungus, *Dermocystidium marinum*, by Mackin *et al.* (1950) and renamed *Labyrinthomyxa marina* by Mackin and Ray (1966), the pathogen was later found by electron microscopy to have protozoan affinities (an apical complex in the zoospore stage) by Perkins (1976b) and was placed by Levine (1978) in the genus *Perkinsus* of the new class Perkinsea of the protozoan phylum Apicomplexa. Consideration of this severe pathogen of oysters is a feature of the next chapter, on protozoan diseases, but it is mentioned here as an example of the taxonomic uncertainties to be found among many protistan parasites of molluscs. Certainly, there are other tissue parasites of bivalves with staining, culture, and ultrastructural characteristics which may warrant their removal from *protista incertae sedis* and their consideration as proper fungi or protozoans. [See, for examples of the possibilities, Perkins (1974, 1976a, Porter (1974), and Quick (1974a).] More is said about these "uncertain" protistans in the next chapter, since a number of bivalve pathogens and potential pathogens can be found here in this "twilight zone" between the Protozoa and the Fungi.

III. CRUSTACEAN FUNGAL DISEASES

Diseases caused by fungi are more prevalent in Crustacea than in any other marine invertebrate group, according to a recent assessment (Sparks, 1985). In this chapter emphasis is placed on several serious mycoses of wild and captive species of economic importance—recognizing that there are also numerous fungal diseases of planktonic and other noneconomic species. Most of the fungal pathogens of the larger Crustacea are phycomycetes, although one ascomycete (*Trichomaris invadens*) and a few members of the Fungi Imperfecti (*Fusarium solani* in particular) are known to cause disease. Information about some of the principal mycoses of crustaceans is summarized in Table 5, and useful reviews of crustacean fungal diseases include those by Johnson (1970), Unestam (1973), Bland (1975), Alderman (1976), Lightner (1981), Johnson (1983), and Sparks (1985). Much of the earlier information about all marine/ estuarine fungi, including the parasitic forms, was discussed in a book by Johnson and Sparrow (1961) and in a subsequent review (Sparrow, 1976).

Several of the fungal diseases of crustaceans that have been described affect the early life history stages: the eggs and larvae. Information about larval diseases, including those of fungal etiology, has accumulated as a consequence of research and development in marine aquaculture, although some has come from observation of wild stocks.

Table 5. Fungal Diseases of Economically Important Marine Crustacea

Host	Disease Name	Pathogen	Disease Signs	Effect on Host	Geographic Distribution	References
<i>Penaeus aztecus</i> , (brown shrimp), <i>Penaeus setiferus</i> (white shrimp); other penaeids may be infected experimentally	<i>Lagenidium</i> disease (larval mycosis)	<i>Lagenidium</i> spp. (especially <i>L. callinectes</i> in North America) and possibly other fungal genera	Inactivity of infected individuals, hyphae visible in appendages and later throughout body	Causes almost total mortality of larvae within 2–3 days	North America and elsewhere in the world	Cook (1971), Lightner (1975, 1977a)
<i>Penaeus japonicus</i> (Kuruma prawn), <i>Penaeus duorarum</i> (pink shrimp), <i>Penaeus californiensis</i> (California brown shrimp)	<i>Fusarium</i> disease of juvenile and adult shrimp	<i>Fusarium solani</i>	Blackened gills containing fungal hyphae, initial focal lesions of damaged tissues which become melanized	May cause mortalities after slow progression; effects variable among host species, with some being refractory to infection	Gulf of Mexico, Pacific coast of Mexico, Japan	Egusa and Ueda (1972), Johnson (1974), Lightner (1977b), Lightner <i>et al.</i> (1979)
<i>Dichelopandalus leptocerus</i>	Gill mycosis	Chytrid-like organism	Gills mottled or darkened, fungus occupies blisterlike area in distal ends of gill lamellae	May interfere with respiration; occurs in high prevalence over wide geographic areas	Northeast coast of the United States	Uzmann and Haynes (1968)
<i>Homarus americanus</i> (American lobster), <i>Homarus gammarus</i> (European lobster)	<i>Haliphthoros</i> disease	<i>Haliphthoros milfordensis</i>	Few to many areas of red-brown melanization at base of walking legs, in gills, and elsewhere	Mortalities occur in juveniles less than 22 mm carapace length; larger animals able to resist in-	Experimental culture facility at Bodega Bay, California	Fisher <i>et al.</i> (1975), Fisher and Nilson (1977)

(continued)

Table 5. (Continued)

Host	Disease Name	Pathogen	Disease Signs	Effect on Host	Geographic Distribution	References
<i>H. americanus</i>	<i>Fusarium</i> disease	<i>Fusarium solani</i>	on exoskeleton; movements stiff, with chelipeds crossed; progressive loss of equilibrium	fections; deaths may result from gill damage, secondary infections, or failure of ecdysis	Experimental rearing facility at Woodside, New York	Lightner and Fontaine (1975)
<i>H. americanus</i> , <i>H. gammarus</i>	<i>Lagenidium</i> disease	<i>Lagenidium</i> sp. (similar to <i>L. callinectes</i>)	Development of exoskeletal lesions in the form of black spots of varying dimensions, gills may be darkened Larvae become opaque white due to proliferation of the fungus	Mortalities occur in juveniles at ecdysis; in one instance mortalities reached 35%	Experimental culture facility at Bodega Bay, California	Nilsson <i>et al.</i> (1976)
<i>Eriocheir sinensis</i> (mitten crab); also found in several European crayfish species	Burn spot disease (<i>Brandfleckenkrankheit</i>)	<i>Septocylindrium eriocheir</i> (<i>Ramularia astaci</i> in the crayfish)	Localized destruction of exoskeleton, forming so-called burn spot	Infections acquired in fresh water, fungus may invade gills and muscles	Germany	Mann (1940), Mann and Pieplow (1938), Unestam (1973)

<i>Chionecetes bairdi</i> (tanner crab)	Black mat disease	<i>Trichomaris invadens</i>	Begins as small black spots on exoskeleton, which enlarge and coalesce; fungal growth produces black tarry mass on carapace, appendages, and eyestalks	Disease is considered lethal, to prevent molting, and to suppress egg production; fungus invades and destroys subepidermal tissue and may invade much of the rest of the body, including connective tissue and muscles	Gulf of Alaska	Van Hyning and Scarborough (1973), Sparks and Hibbits (1979), Hibbits <i>et al.</i> (1981), Mix and Sparks (1980), Sparks (1982a,b), Hicks (1982)
<i>Callinectes sapidus</i> (blue crab); may also infect eggs of other crabs— <i>Panopeus herbstii</i> and <i>Libinia du-bia</i> —as well as the eggs of the barnacle <i>Chelonibia patula</i>	"Sponge" disease	<i>Lagenidium callinectes</i>	Shiny gray to black patches on egg masses (sponges) of crabs	Fungus may destroy 25% of eggs in sponge; may also infect and kill zoea larvae	East coast of the United States	Couch (1942), Sandoz <i>et al.</i> (1944), Rogers-Talbert (1948), Johnson and Bonner (1960), Bland and Amerson (1974)

Eggs of blue crabs from lower Chesapeake Bay were found to be parasitized by a phycomycetous fungus, *Lagenidium callinectes* (Couch, 1942; Sandoz *et al.*, 1944; Newcombe and Rogers, 1947; Rogers-Talbert, 1948; Bland and Amerson, 1973, 1974). Infected eggs either failed to hatch or gave rise to abnormal zoea larvae. Up to 90% of a sample of ovigerous female crabs were infected, and up to 25% of the eggs in a "sponge" (egg mass) were invaded. Penetration of the egg mass was slow and did not exceed 3 mm. This fact, combined with the short (2-week) incubation time, permitted normal development of much of the egg mass internal to the infection. Experimentally, the fungus developed normally in salinities from 5 to 30 ‰ and also killed blue crab larvae. The fungus was transmitted experimentally to the eggs of two other species of crabs (the oyster crab and the mud crab, *Neopanope texiana*) inhabiting the same bay area.

Lagenidium subsequently has been found to infect larvae of the American lobster (Nilson *et al.*, 1976; Fisher *et al.*, 1976), various species of penaeid shrimp (Lightner and Fontaine, 1973; Lightner, 1977a) (Fig. 10), the Dungeness crab (Armstrong *et al.*, 1976), and the mangrove crab (Bian *et al.*, 1977). It also occurs in the eggs and larvae of barnacles and brine shrimp (Lightner, 1981).

Lagenidium has proved to be a problem in crustacean culture, where it may cause epizootics and mortalities of eggs and larvae of penaeid



Figure 10. Larval shrimp (*Penaeus setiferus*) with advanced *Lagenidium callinectes* infection. (Courtesy of Dr. D. V. Lightner.)

shrimp, lobsters, and Dungeness crabs. In lobsters the surface layers of egg masses may be infected, just as in the blue crabs, with a similar slow rate of progression (Fisher *et al.*, 1978). Effects on lobster larvae may be severe, with mortality rates in excess of 90% (Nilson *et al.*, 1976); high larval mortality rates also characterized infections of Dungeness crabs and shrimp (Armstrong *et al.*, 1976; Lightner, 1977a). Progression of the disease in crustacean larvae has been examined thoroughly. Infective zoospores settle and penetrate the exoskeleton; hyphae spread throughout appendages and body musculature, causing extensive tissue necrosis, immobilization, and death; spore discharge tubes form after death of the host, and release motile zoospores (Lightner, 1985).

A closely related phycomycete, *Sirolopidium* sp., has been reported to infect penaeid shrimp larvae, with disease progression and mortality rates similar to those of *L. callinectes* (Lightner, 1977a).

Still another phycomycetous fungus, *Haliphthoros milfordensis*, described earlier in this chapter as a pathogen of gastropod molluscs (abalone and oyster drills), also infects penaeid shrimp, lobsters, and blue crabs (Tharp and Bland, 1977). The disease is characterized by penetration through thin, uncalcified parts of the exoskeleton, followed by tissue destruction, especially in gills and eyes. Invasion elicits a strong host inflammatory response, with encapsulation and melanization of hyphae, and formation of adhesions which interfere with molting (Fisher *et al.*, 1975, 1976, 1978). Experimental mortalities have been produced in shrimp and postlarval lobsters in 5–22 days. Another species of *Haliphthoros*, *H. philippinensis*, has been isolated recently from larvae of the tiger prawn, *Penaeus monodon*, being reared in the Philippines (Hatai *et al.*, 1980).

A mycosis affecting English prawns was described in some detail by Anderson and Conroy (1968). The disease seemed to be associated with damage to the exoskeleton and was characterized by massive fungal invasion of the hemocoel, muscles, and sometimes the gonads. The course of infection was rapid, and mortalities occurred in culture tanks. Isolates were tentatively identified as *Pythium* sp., and transmission was effected by inoculation and feeding.

Other phycomycetous fungi affect crustaceans. Pea crabs (*Pinnotheres pisum*) taken from the sea mussel at Plymouth, England, were parasitized by the fungus *Leptolegnia marina* (Atkins, 1929, 1954a). The mycelium was usually found in the gills, but penetrated other body organs and appendages as well. Zoosporangia developed in the appendages, and large numbers of zoospores were released upon death of the host. No further growth of the fungus took place in dead crabs, and no external development, beyond papillae of zoospore exit tubes, was seen.

Atkins (1954b, 1955) described two other fungi, *Plectospora dubia* and *Pythium thalassium*, which infect eggs of pea crabs and other Crustacea. *Plectospora dubia* (now *Atkinsiella dubia*) was less pathogenic for prezoal and zoal stages than for earlier stages of egg development. *Atkinsiella dubia* has been reported subsequently as infecting egg masses of seven genera of crabs from the northwest Pacific coast of the United States and could be considered a potentially serious pathogen of crustacean eggs (Lightner, 1981).

Two crustacean diseases have been attributed to chytrid-like fungi. One was described from feral populations of pandalid shrimp, *Dichelopandalus leptocerus*, from the continental shelf of the northeast coast of United States (Uzmann and Haynes, 1968). The chytrid-like parasite—not further identified—invaded the gills, producing a dark brown discoloration. Diseased shrimp were found at 79 of 126 sampling locations from southern Nova Scotia to Long Island, and the prevalence in individual samples reached 96%. The authors suggested that the infection could have serious effects on the respiratory efficiency of diseased individuals and that it could be an important mortality factor in this and other species of shrimp. A similar “black spot” pigmentation of the gills of another pandalid shrimp, *Pandalus borealis*, was reported from the same geographic area, but no pathogen was identified (Rinaldo and Yevich, 1974).

Another presumptive fungal disease, caused by an organism with possible chytrid-like affinities, is the so-called “mottling disease” of American lobsters from the Maine coast. Characterized by yellowish splotches in an otherwise dark green exoskeleton (Fig. 11), the condition has been known for many years as a color variation. Affected individuals are called “leopard lobsters” (Herrick, 1895, 1911). The shell condition and color result from progressive growth of areas of necrosis in underlying tissues and, in advanced cases, even blisters of the shell. The areas of necrosis expand slowly in lobsters held in seawater tanks. Histological examination of diseased tissues disclosed numerous Schiff-positive, subspherical, heavy-walled bodies, 30–60 μm in diameter. Preliminary attempts at culture and transmission were unsuccessful. The disease occurs infrequently in Gulf of Maine lobster populations and has not been reported from other areas (Sindermann, 1970).

A few representatives of other broad taxa of fungi (other than the Phycomycetes) may be pathogenic for marine Crustacea. One with potentially serious consequences is black mat disease of tanner crabs, *Chionoecetes bairdi*, caused by an ascomycete, *Trichomarix invadens* (Sparks and Hibbits, 1979; Hibbits *et al.*, 1981). The disease is apparent because of black surface encrustation, but the fungus penetrates the exoskeleton and



Figure 11. Mottling disease of the lobster caused by a presumptive fungus pathogen.

invades hypodermal and other internal tissues, causing extensive destruction (Sparks, 1982a). Infection prevalences of up to 75% were seen in some samples from Alaskan waters (Van Hyning and Scarborough, 1973), but mortalities due to the disease have not been reported, even though interference with molting probably occurs even in moderately infected individuals (Sparks, 1982b, 1985). An interesting aspect of the disease is the absence of host inflammatory response—suggesting an acute lethal disease, despite lack of evidence of mortalities. Sparks (1985) postulated that *T. invadens* infection was a major factor in recent declines in tanner crab populations.

Several members of the Fungi Imperfecti have also been described as pathogens of Crustacea. Prominent among them is *Fusarium solani* (and possibly other *Fusarium* spp.) identified as a pathogen of at least eight species of penaeid shrimp as well as freshwater prawns and American lobsters (Hatai and Egusa, 1978; Burns *et al.*, 1979; Alderman, 1981; Lightner, 1981). Prominent features of *Fusarium* disease are blackened exoskeletal spots, black gills, and penetration of underlying tissues (Fig. 12)—accompanied by pronounced inflammatory reaction and extensive tissue destruction. It is known variously as black spot disease or black gill disease, and is usually subacute but progressive, eventually leading to death of the infected animal. Among penaeid shrimp, susceptibility varies with species and life cycle stage (Lightner and Fontaine, 1978, 1975; Lightner, 1976; Lightner *et al.*, 1979; Hose *et al.*, 1984), and some strains of *F. solani* produce a mycotoxin pathogenic to shrimp. Among

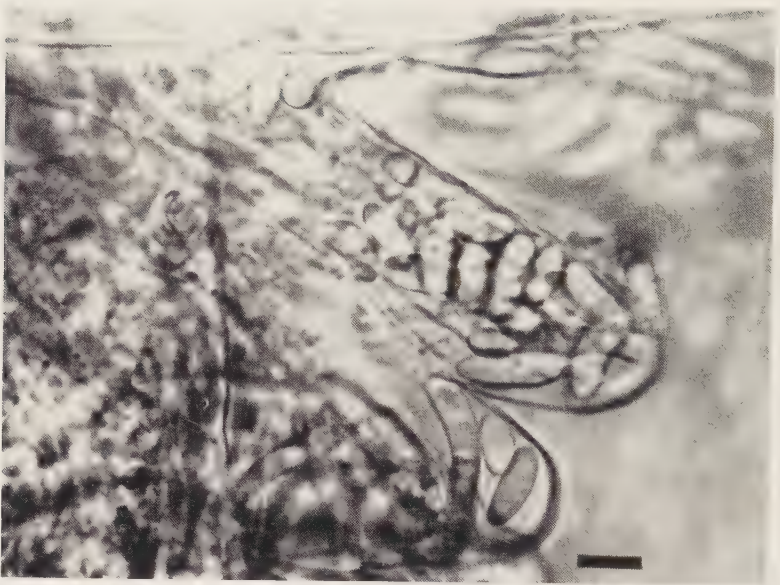


Figure 12. *Fusarium* infection of gills of cultured shrimp. Note typical conidiospores of the fungus. Bar, 10 μm . (Courtesy of Dr. D. V. Lightner.)

juvenile lobsters, individuals with extensive black spots characteristic of *Fusarium* disease died just before or during molting (Lightner and Fontaine, 1975); larvae were not infected.

A fungal disease of European lobsters, *Homarus gammarus*, and spiny lobsters, *Palinurus vulgaris*, was associated with mortalities in the public aquarium at Livorno in Italy by Sordi (1958). Two fungi, *Fusarium* (= *Ramularia*) *branchialis* and the closely related *Didymaria palinuri*—both Fungi Imperfecti—were isolated from extensive gill lesions, but *F. branchialis* was considered to be the principal pathogen. Subsequently, another similar fungus was observed in *P. vulgaris*, this time from the Irish Sea. The animals exhibited a black spot condition in high prevalences, but the pathogen was not identified (Alderman, 1971, 1973, 1976).

These then are the principal fungal diseases of economically important marine crustaceans—mostly phycomycetes, a few Fungi Imperfecti, and one ascomycete. Additionally, an interesting and aberrant group of parasitic or commensal fungi, the trichomycetes, occurs on the lining of the gut or on the exoskeleton of Crustacea, particularly amphipods and isopods. A number of species have been described from various crabs, especially the hermit crabs. These fungi require a chitinous substrate,

and exhibit specificity not only for particular host species, but for location in or on the host as well. Furthermore, spore formation in some instances seems to be linked with molting of the host. Duboscq *et al.* (1949) and Manier (1950) have summarized information about the group, pointing out a lack of demonstrated pathogenicity.

In summary, fungal infections are common in marine Crustacea, but most appear to result from opportunistic invasions of injured or stressed hosts. Eggs and larvae seem to be the most vulnerable, and recent studies in aquaculture environments have disclosed fungal epizootics and mass mortalities of early life history stages—with phycomycetes being principal culprits. Genera prominent as pathogens of cultured crustaceans (i.e., shrimp, American lobster, blue crab, Dungeness crab) include *Lagenidium* and *Haliphthoros*—as well as *Fusarium* of the Fungi Imperfecti. Thus far, no major primary fungal pathogen comparable to *Aphanomyces astaci*, which causes the widespread freshwater crayfish plague in Europe, has been observed in marine crustaceans of economic importance—although reported epizootics and mass mortalities in zooplankton populations are matters of concern (Vallin, 1951; Höhnk and Vallin, 1953; Zelezinskaya, 1972; Unestam, 1973; Johnson, 1983).

IV. CONCLUSIONS

The relatively sparse literature on fungal diseases of economically important marine shellfish discloses no virulent primary pathogen comparable to the bacterium *Aerococcus viridans* in lobsters. What is revealed is a heterogeneous assemblage of mostly phycomycetous organisms which may destroy developing crustacean eggs, and which may also occur sporadically at epizootic levels in molluscan and crustacean larvae being reared in shellfish hatcheries. Only one fungal disease of shellfish—shell disease of European oysters, caused by *Ostracoblabe implexa*—has occurred in outbreak proportions and has been reported to cause mass mortalities in wild as well as cultured stocks. Other than these examples—diseases of eggs and larvae and shell disease of oysters—the record is one of isolated appearances of fungi as the cause of black mat disease of tanner crabs, black spot disease of English prawns and mitten crabs in Europe, and black gill disease in captive European lobsters. A reservoir of potential pathogens exists, however, in an amorphous mass of tissue parasites seen and occasionally reported in molluscan shellfish. Some of these organisms, now placed in *protista incertae sedis*, mostly because of the lack of study, may well be described eventually as fungi, and a pathogenic role may be identified.

The fungi are better represented among the smaller Crustacea, and epizootics have been reported in planktonic and benthic populations. Fungi also seem more abundant in freshwater crustaceans, in which numerous outbreaks have been described—with the “pathogen of the century” award going to *Aphanomyces astaci*, which causes European crayfish plague.

REFERENCES

- Alderman, D. J. (1971). Shellfish disease in the United Kingdom 1970–71. *Int. Counc. Explor. Sea, Doc. C.M.1971/K:19*.
- Alderman, D. J. (1973). Fungal infection of crawfish (*Palinurus elephas*) exoskeleton. *Trans. Br. Mycol. Soc.* **61**, 595–597.
- Alderman, D. J. (1976). Fungal diseases of marine animals. In “Recent Advances in Aquatic Mycology” (E. B. G. Jones, ed.), pp. 223–260. Wiley, New York.
- Alderman, D. J. (1981). *Fusarium solani* causing an exoskeletal pathology in cultured lobsters, *Homarus vulgaris*. *Trans. Br. Mycol. Soc.* **76**, 25–27.
- Alderman, D. J. (1986). Fungal diseases of marine invertebrates. *Eur. Aquacult. Soc., Spec. Publ.* **9**, 197–201.
- Alderman, D. J., and Jones, E. B. G. (1967). Shell disease of *Ostrea edulis* L. *Nature (London)* **216**, 797–798.
- Alderman, D. J., and Jones, E. B. G. (1971a). Shell disease of oysters. *Fish. Invest. (London), Ser. 2* **26**(8), 1–19.
- Alderman, D. J., and Jones, E. B. G. (1971b). Physiological requirements of two marine phycomycetes, *Althornia crouchii* and *Ostracoblabe implexa*. *Trans. Br. Mycol. Soc.* **57**, 213–225.
- Alderman, D. J., Harrison, J. L., Bremer, G. B., and Jones, E. B. G. (1974). Taxonomic revisions in the marine biflagellate fungi: The ultrastructural evidence. *Mar. Biol. (Berlin)* **25**, 345–357.
- Anderson, J. I. W., and Conroy, D. A. (1968). The significance of disease in preliminary attempts to raise Crustacea in seawater. *Bull. Off. Int. Epizoot.* **69**, 1239–1249.
- Armstrong, D. A., Buchanan, D. V., and Caldwell, R. S. (1976). A mycosis caused by *Lagenidium* sp. in laboratory-reared larvae of the Dungeness crab, *Cancer magister*, and possible chemical treatments. *J. Invertebr. Pathol.* **28**, 329–336.
- Atkins, D. (1929). On a fungus allied to the Saprolegniaceae found in the pea-crab *Pinnotheres*. *J. Mar. Biol. Assoc. U.K.* **16**, 203–219.
- Atkins, D. (1954a). Further notes on a marine member of the Saprolegniaceae, *Leptolegnia marina* n. sp., infecting certain invertebrates. *J. Mar. Biol. Assoc. U.K.* **33**, 613–625.
- Atkins, D. (1954b). A marine fungus *Plectospora dubia* n. sp. (Saprolegniaceae), infecting crustacean eggs and small Crustacea. *J. Mar. Biol. Assoc. U.K.* **33**, 721–732.
- Atkins, D. (1955). *Pythium thalassium* n. sp. infecting the egg-mass of the pea-crab *Pinnotheres pisum*. *Trans. Br. Mycol. Soc.* **38**, 31–46.
- Bian, B. Z., Hatai, K., Po, G. L., and Egusa, S. (1977). Studies on the fungal diseases in crustaceans. I. *Lagenidium scyllae* sp. nov. isolated from cultured ova and larvae of the mangrove crab (*Scylla serrata*). *Trans. Mycol. Soc. Jpn.* **20**, 115–124.
- Bland, C. E. (1975). Fungal diseases of marine Crustacea. *Proc. U.S.–Jpn. Meet. Aquacult.* **3rd** pp. 41–48.
- Bland, C. E., and Amerson, H. V. (1973). Observations on *Lagenidium callinectes* Couch: Isolation and sporangial development. *Mycologia* **65**, 310–320.

- Bland, C. E., and Amerson, H. V. (1974). Occurrence and distribution in North Carolina waters of *Lagenidium callinectes* Couch, a fungal parasite of blue crab ova. *Chesapeake Sci.* **15**, 232–235.
- Bornet, E., and Flahault, C. (1889). Sur quelques plantes vivant dans le test calcaire des mollusques. *Bull. Soc. Bot. Fr.* **36**, 147–176.
- Bower, S. M. (1987a). *Labyrinthuloides haliotidis* n. sp. (Protozoa: Labyrinthomorpha), a pathogenic parasite of small juvenile abalone in a British Columbia mariculture facility. *Can. J. Zool.* **65**, 1996–2007.
- Bower, S. M. (1987b). Pathogenicity and host specificity of *Labyrinthuloides haliotidis* (Protozoa: Labyrinthomorpha), a parasite of juvenile abalone. *Can. J. Zool.* **65**, 2008–2012.
- Bower, S. M. (1987c). Artificial culture of *Labyrinthuloides haliotidis* (Protozoa: Labyrinthomorpha), a pathogenic parasite of abalone. *Can. J. Zool.* **65**, 2013–2020.
- Bower, S. M. (1987d). The life cycle and ultrastructure of a new species of thraustochytrid (Protozoa: Labyrinthomorpha) pathogenic to small abalone. *Aquaculture* **67**, 269–272.
- Burns, C. D., Berrigan, M. E., and Henderson, G. E. (1979). *Fusarium* sp. infections in the freshwater prawn *Macrobrachium rosenbergii* (DeMan). *Aquaculture* **16**, 193–198.
- Cole, H. A. (1950). Shell disease in relaid French oysters. *Nature (London)* **166**, 19–20.
- Cole, H. A., and Hancock, D. A. (1956). Progress in oyster research in Britain 1949–1954, with special reference to the control of pests and diseases. *Rapp. P.V. Reun., Cons. Int. Explor. Mer* **140**, 24–29.
- Cole, H. A., and Waugh, G. D. (1956). Shell disease in Portuguese oysters. *Nature (London)* **178**, 422.
- Cook, H. L. (1971). Fungi parasitic on shrimp. *FAO Aquacult. Bull.* **3**(4), 13.
- Couch, J. N. (1942). A new fungus on crab eggs. *J. Elisha Mitchell Sci. Soc.* **58**, 158–162.
- Davis, H. C., Loosanoff, V. L., Weston, W. H., and Martin, C. (1954). A fungus disease in clam and oyster larvae. *Science* **120**, 36–38.
- Dollfus, R. P. (1921). Résumé de nos principales connaissances pratiques sur les maladies et les ennemis de l'huître. *Notes Mem. Off. Sci. Tech. Pêche Marit.* **7**, 1–58.
- Dollfus, R. P. (1923). La maladie des moules et la mortalité des huîtres Zélande au cours de ces dernières années. *Bull. Soc. Cent. Aquic. Pêche* **30**, 38–44.
- Duboscq, O., Leger, L., and Tuzet, O. (1949). Contribution à la connaissance des éccrinides. Les trichomycètes. *Arch. Zool. Exp. Gen.* **86**, 29–144.
- Durve, V. S., and Bal, D. V. (1960). Shell disease in *Crassostrea gryphoides* (Schlotheim). *Curr. Sci.* **20**, 489–490.
- Egusa, S., and Ueda, T. (1972). A *Fusarium* sp. associated with black gill disease of the Kuruma prawn, *Penaeus japonicus* Bate. *Nippon Suisan Gakkaishi* **38**, 1253–1260.
- Fisher, W. S., and Nilson, E. H. (1977). *Haliphthoros* disease of lobsters. In "Disease Diagnosis and Control in North American Marine Aquaculture. Developments in Aquaculture and Fisheries Science" (C. J. Sindermann, ed.), pp. 173–177. Elsevier, Amsterdam.
- Fisher, W. S., Nilson, E. H., and Shleser, R. A. (1975). Effect of the fungus *Haliphthoros milfordensis* on the juvenile stages of the American lobster, *Homarus americanus*. *J. Invertebr. Pathol.* **26**, 41–45.
- Fisher, W. S., Nilson, E. H., Follett, L. F., and Shleser, R. A. (1976). Hatching and rearing lobster larvae (*Homarus americanus*) in a disease situation. *Aquaculture* **7**, 75–80.
- Fisher, W. S., Nilson, E. H., Steenbergen, J. F., and Lightner, D. V. (1978). Microbial diseases of cultured lobsters: A review. *Aquaculture* **14**, 115–140.
- Gaertner, A. (1972). Characters used in the classification of thraustochytriaceous fungi. *Veroeff. Inst. Meeresforsch. Bremerhaven* **13**, 183–194.
- Galtsoff, P. S. (1964). The American oyster *Crassostrea virginica* Gmelin. *U.S. Fish Wildl. Serv., Fish. Bull.* **64**, 1–480.

- Galtsoff, P. S. (1969). Anomalies and malformations in the shells of *Crassostrea virginica*. *Natl. Cancer Inst. Monogr.* **31**, 575–580.
- Ganaros, A. E. (1957). Marine fungus infecting eggs and embryos of *Urosalpinx cinerea*. *Science* **125**, 1194.
- Giard, A. (1894). Sur une affection parasitaire de l'huître (*Ostrea edulis* L.) connue sous le nom de maladie du pied. *C. R. Soc. Biol.* **46**, 401–403.
- Hatai, K. (1982). On the fungus *Haliphthoros milfordensis* isolated from temporarily held abalone (*Haliotis sieboldii*). *Gyobyo Kenkyu* **17**, 199–204. (in Japanese; English abstract).
- Hatai, K., and Egusa, S. (1978). Studies on the pathogenic fungus with black gill disease of kuruma prawn, *Penaeus japonicus*: II. Some notes on BG-*Fusarium*. *Gyobyo Kenkyu* **12**, 225–232 (in Japanese).
- Hatai, K., Bian, B. Z., Baticadas, M. C. L., and Egusa, S. (1980). Studies on the fungal diseases in crustaceans. II. *Haliphthoros philippinensis* sp. nov. isolated from cultivated larvae of the jumbo tiger prawn (*Penaeus monodon*). *Trans. Mycol. Soc. Jpn.* **21**, 47–55.
- Havinga, B. (1931). Contribution to the knowledge of oyster diseases. *Rapp. Groupe I, Sect. 8, Congr. Int. Agric. Pêches* 1931, 61 pp.
- Herrick, F. H. (1895). The American lobster; a study of its habits and development. *Bull. U.S. Fish Comm.* **15**, 1–252.
- Herrick, F. H. (1911). Natural history of the American lobster. *U.S. Bur. Fish. Bull.* **29**, 149–408.
- Hibbits, J., Hughes, G. C., and Sparks, A. K. (1981). *Trichomarix invadens* gen. et sp. nov., an ascomycete parasite of the tanner crab (*Chionoecetes bairdi* Rathbun (Crustacea: Brachyura). *Can. J. Bot.* **59**, 2121–2128.
- Hicks, D. M. (1982). Abundance and distribution of black mat syndrome on stocks of tanner crabs, *Chionoecetes bairdi*, in the northwestern Gulf of Alaska. *Proc. Int. Symp. Genus Chionoecetes, Univ. Alaska, Alaska Sea Grand Rep.* **82-10**, 563–579.
- Hirsch, C. A. E. (1921). Über eine Muschelkrankheit in Holland. *Fischerbote* **13**, 217–223.
- Höhnk, W., and Vallin, S. (1953). Epidemisches Absterben von *Eurytemora* im Bottnischen Meerbusen, verursacht durch *Leptolegnia baltica* nov. spec. *Veroeff. Inst. Meeresforsch. Bremerhaven* **2**, 215–223.
- Hornell, J. (1910). The practice of oyster culture at Arcachon and its lessons for India. *Madras Fish. Bull.* **2**(4), 1–90.
- Hose, J. E., Lightner, D. V., Redman, R. M., and Danald, D. A. (1984). Observations on the pathogenesis of the imperfect fungus, *Fusarium solani*, in the California brown shrimp, *Penaeus californiensis*. *J. Invertebr. Pathol.* **44**, 292–303.
- Johnson, P. T. (1983). Diseases caused by viruses, rickettsiae, bacteria, and fungi. In "The Biology of the Crustacea" (A. Provenzano, ed.), Vol. 6, pp. 1–78. Academic Press, New York.
- Johnson, S. K. (1974). *Fusarium* sp. in laboratory held pink shrimp. *Tex. Agric. Ext. Serv., Fish Dis. Diagn. Lab. FDDL-51*, 1 p.
- Johnson, T. W., Jr. (1970). Fungi in marine crustaceans. In "A Symposium on Disease of Fishes and Shellfishes" (S. F. Snieszko, ed.), Spec. Publ., pp. 405–408. Am. Fish. Soc., Washington, D.C.
- Johnson, T. W., Jr. and Bonner, R. R., Jr. (1960). *Lagenidium callinectes* Couch in barnacle ova. *J. Elisha Mitchell Sci. Soc.* **76**, 147–149.
- Johnson, T. W., Jr., and Sparrow, F. K., Jr. (1961). "Fungi in Oceans and Estuaries," 668 pp. Hafner, New York.
- Jones, G. M., and O'Dor, R. K. (1983). Ultrastructural observations on a thraustochytrid fungus parasitic in the gills of squid (*Illex illecebrosus* Le Sueuer). *J. Parasitol.* **69**, 903–911.
- Korringa, P. (1947). Les vicissitudes de l'ostréiculture Hollandaise élucidées par la science ostréicole moderne. *Ostréicult., Cult. Mar.* **16**, 3–9.

- Korringa, P. (1948). Shell disease in *Ostrea edulis*—Its dangers, its cause, its control. *Conv. Addresses Natl. Shellfish. Assoc.* 1947 pp. 89–94.
- Korringa, P. (1951a). On the nature and function of “chalky” deposits in the shell of *Ostrea edulis* Linnaeus. *Proc. Calif. Acad. Sci.* **27**, 133–158.
- Korringa, P. (1951b). Investigations on shell-disease in the oyster, *Ostrea edulis* L. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **128**, 50–54.
- Korringa, P. (1952). Recent advances in oyster biology. *Q. Rev. Biol.* **27**, 266–308 and 339–365.
- Lauckner, G. (1983). Diseases of Mollusca: Bivalvia. In “Diseases of Marine Animals” (O. Kinne, ed.), Vol. II, pp. 477–961. Biol. Anstalt Helgol., Hamburg, FRG.
- Levine, N. D. (1978). *Perkinsus* gen. n. and other new taxa in the protozoan phylum Apicomplexa. *J. Parasitol.* **64**, 549.
- Levine, N. D., Corliss, J. O., Cox, F. E. G., Deroux, G., Grain, J., Honigberg, B. M., Leedale, G. F., Loeblich, A. R., III, Lom, J., Lynn, D., Merinfeld, E. G., Page, F. C., Poljansky, G., Sprague, V., Vavra, J., and Wallace, F. G. (1980). A newly revised classification of the Protozoa. *J. Protozool.* **27**, 37–58.
- Lightner, D. V. (1975). Some potentially serious disease problems in the culture of penaeid shrimp in North America. *Proc. U.S.-Jpn. Meet. Aquacult.* 3rd pp. 75–97.
- Lightner, D. V. (1976). Epizootiology of two mycotic diseases in the culture of penaeid shrimp. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st pp. 179–183.
- Lightner, D. V. (1977a). Larval mycosis of shrimps. In “Disease Diagnosis and Control in North American Marine Aquaculture” (C. J. Sindermann, ed.), pp. 36–41. Elsevier, Amsterdam.
- Lightner, D. V. (1977b). Fungus (*Fusarium*) disease of shrimps. In “Disease Diagnosis and Control in North American Aquaculture” (C. J. Sindermann, ed.), pp. 42–47. Elsevier, Amsterdam.
- Lightner, D. V. (1981). Fungal diseases of marine Crustacea. In “Pathogenesis of Invertebrate Microbial Diseases” (E. W. Davidson, ed.), pp. 451–484. Allanheld & Osmun, Totowa, New Jersey.
- Lightner, D. V. (1985). A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. *Proc. Int. Conf. Cult. Penaeid Prawns/Shrimps*, 1st, pp. 79–103.
- Lightner, D. V., and Fontaine, C. T. (1973). A new fungus disease of the white shrimp, *Penaeus setiferus*. *J. Invertebr. Pathol.* **22**, 94–99.
- Lightner, D. V., and Fontaine, C. T. (1975). A mycosis of the American lobster, *Homarus americanus*, caused by *Fusarium* sp. *J. Invertebr. Pathol.* **25**, 239–245.
- Lightner, D. V., Moore, D., and Danald, D. A. (1979). A mycotic disease of cultured penaeid shrimp caused by the fungus *Fusarium solani*. *Tex. A&M Univ. Sea Grant Program [Rep.] TAMU-SG-79-114*, 135–158.
- Loosanoff, V. L. (1954). New advances in the study of bivalve larvae. *Am. Sci.* **42**, 607–624.
- Loosanoff, V. L. (1974). Factors responsible for the mass mortalities of molluscan larvae in nature: A review. *Proc. Annu. Meet.—World Maric. Soc.* **5**, 297–309.
- Loosanoff, V. L. and Davis, H. C. (1963). Rearing of bivalve mollusks. *Adv. Mar. Biol.* **1**, 1–136.
- Mackin, J. G. and Ray, S. M. (1966). The taxonomic relationships of *Dermocystidium marinum* Mackin, Owen, and Collier. *J. Invertebr. Pathol.* **8**, 544–545.
- Mackin, J. G., Owen, H. M., and Collier, A. (1950). Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp., in *Crassostrea virginica* (Gmelin). *Science* **111**, 328–329.
- Manier, J. F. (1950). Recherches sur les trichomycètes. *Ann. Sci. Nat., Bot. Biol. Veg.* **11**, 53–162.

- Mann, H. (1940). Die Brandfleckenkrankheit beim Sumpfkrebs (*Potamobius leptodactylus* Esch.). *Z. Parasitenkd.* **11**, 430–432.
- Mann, H. and Pieplow, U. (1938). Die Brandfleckenkrankheit bie Krebsen und ihre Erreger. *Z. Fisch. Deren Hilfswiss.* **36**, 225–240.
- McLean, N., and Porter, D. (1982). The yellow spot disease of *Tritonia diomedea* Bergh. (Mollusca: Gastropoda: Nudibranchia): Encapsulation of the thraustochytriacous parasite by host amoebocytes. *J. Parasitol.* **68**, 243–252.
- Mix, M. C., and Sparks, A. K. (1980). Tanner crab, *Chionoecetes bairdi* Rathbun haemocyte classification and an evaluation of using differential counts to measure infection with a fungal disease. *J. Fish Dis.* **3**, 285–293.
- Montagne, F. (1878). Études pratiques sur les ennemies et les maladies de l'huître dans le bassin d'Arcachon. *Actes Soc. Linn. Bordeaux* **32**, 217–245.
- Moss, S. T. (1985). An ultrastructural study of taxonomically significant characters of the Thraustochytriales and the Labyrinthulales. *Bot. J. Linn. Soc.* **91**, 329–357.
- Newcombe, C. L., and Rogers, M. R. (1947). Studies of a fungus parasite that infects blue crab eggs. *Turtlex News* **25**, 1–7.
- Nilson, E. H., Fisher, W. S., and Shleser, R. A. (1976). A new mycosis of larval lobster (*Homarus americanus*). *J. Invertebr. Pathol.* **27**, 177–183.
- Olive, L. S. (1975). "The Mycetozoans." Academic Press, New York.
- Orton, J. H. (1937). "Oyster Biology and Oyster Culture," Buckland Lectures for 1935, No. 3, 211 pp. Arnold, London.
- Perkins, F. O. (1974). Phylogenetic considerations of the problematic thraustochytriacous–labyrinthulid–*Dermocystidium* complex based on observations of fine structure. *Veroeff. Inst. Meeresforsch. Bremerhaven, Suppl.* **5**, 45–63.
- Perkins, F. O. (1976a). Fine structure of lower marine and estuarine fungi. In "Recent Advances in Aquatic Mycology" (E. B. G. Jones, ed.), pp. 279–312. Elek Sci., London.
- Perkins, F. O. (1976b). Zoospores of the oyster pathogen, *Dermocystidium marinum*. I. Fine structure of the conoid and other sporozoan-like organelles. *J. Parasitol.* **62**, 959–974.
- Pokorny, K. S. (1985). Phylum Labyrinthomorpha. In "An Illustrated Guide to the Protozoa" (J. J. Lee, S. H. Hutner, and E. C. Bovee, eds.), pp. 318–321. Soc. Protozool., Lawrence, Kansas.
- Polglase, J. L. (1980). A preliminary report on the thraustochytrid(s) and labyrinthulid(s) associated with a pathological condition in the lesser octopus *Eledone cirrhosa*. *Bot. Mar.* **23**, 699–706.
- Porter, D. (1974). Phylogenetic considerations of the Thraustochytriaceae and Labyrinthulaceae. *Veroeff. Inst. Meeresforsch. Bremerhaven, Suppl.* **5**, 19–44.
- Quick, J. A. (1972a). Fluid thioglycollate medium assay of *Labyrinthomyxa* parasites in oysters. *Leaflet. Ser.—Fla., Dep. Nat. Resour., Mar. Res. Lab.* **6** (Part 4, No. 3), 1–12.
- Quick, J. A. (1972b). A new thraustochytridaceous fungus endoparasitic to the American oyster, *Crassostrea virginica* (Gmelin), in Florida. *Newslet. Soc. Invertebr. Pathol.* **4**(3), 13.
- Quick, J. A. (1974a). *Labyrinthuloides schizochytrrops* n. sp., a new marine labyrinthula with spheroid spindle cells. *Trans. Am. Microsc. Soc.* **93**, 344–365.
- Quick, J. A. (1974b). A new marine *Labyrinthula* with unusual locomotion. *Trans. Am. Microsc. Soc.* **93**, 52–61.
- Raghukumar, C., and Lande, V. (1988). Shell disease of rock oyster *Crassostrea cucullata*. *Dis. Aquat. Org.* **4**, 77–81.
- Rinaldo, R. G., and Yevich, P. (1974). Black spot gill syndrome of the northern shrimp *Pandalus borealis*. *J. Invertebr. Pathol.* **24**, 224–233.
- Rogers-Talbert, R. (1948). The fungus *Lagenidium callinectes* Couch (1942) on eggs of the blue crab in Chesapeake Bay. *Biol. Bull. (Woods Hole, Mass.)* **95**, 214–228.

- Sandoz, M. D., Rogers, M. R., and Newcombe, C. L. (1944). Fungus infection of eggs of the blue crab, *Callinectes sapidus* Rathbun. *Science* **99**, 124–125.
- Sindermann, C. J. (1970). "Principal Diseases of Marine Fish and Shellfish," 369 pp. Academic Press, New York.
- Sordi, M. (1958). Micosi dei Crostacei decapodi marini. *Riv. Parassitol.* **19**, 131–137.
- Sparks, A. K. (1982a). Observations on the histopathology and probable progression of the disease caused by *Trichomaris invadens*, an invasive ascomycete, in the tanner crab, *Chionoecetes bairdi*. *J. Invertebr. Pathol.* **40**, 242–254.
- Sparks, A. K. (1982b). The histopathology and possible role in the population dynamics of the tanner crab, *Chionoecetes bairdi*, of the fungus disease (black mat syndrome) caused by *Trichomaris invadens*. *Proc. Int. Symp. Genus Chionoecetes, Univ. Alaska, Alaska Sea Grant Rep.* **82–10**, 539–546.
- Sparks, A. K. (1985). "Synopsis of Invertebrate Pathology Exclusive of Insects." Elsevier, Amsterdam.
- Sparks, A. K., and Hibbits, J. (1979). Black mat syndrome, an invasive mycotic disease on the tanner crab, *Chionoecetes bairdi*. *J. Invertebr. Pathol.* **34**, 184–191.
- Sparrow, F. K. (1976). The present status of classification in biflagellate fungi. In "Recent Advances in Aquatic Mycology" (E. B. Gareth-Jones, ed.), pp. 213–222. Elek Sci., London.
- Sprague, V. (1971). Diseases of oysters. *Annu. Rev. Microbiol.* **25**, 211–230.
- Tharp, T. P., and Bland, C. E. (1977). Biology and host range of *Haliphthoros milfordensis*. *Can. J. Bot.* **55**, 2936–2944.
- Unestam, T. (1973). Fungal diseases of Crustacea. *Rev. Med. Vet. Mycol.* **8**, 1–20.
- Uzmann, J. R., and Haynes, E. B. (1968). A mycosis of the gills of the pandalid shrimp, *Dichelopandalus leptoceros* (Smith). *J. Invertebr. Pathol.* **12**, 275–277.
- Vallin, S. (1951). Plankton mortality in the northern Baltic caused by a parasitic water-mould. *Annu. Rep. Inst. Freshwater Res. Drottningholm* **32**, 139–148.
- Van Hying, J. M., and Scarborough, A. M. (1973). Identification of fungal encrustation on the shell of the snow crab (*Chionoecetes bairdi*). *J. Fish. Res. Board Can.* **30**, 1738–1739.
- Vishniac, H. S. (1955). The morphology and nutrition of a new species of *Sirolopidium*. *Mycologia* **47**, 633–645.
- Vishniac, H. S. (1958). A new marine Phycomycete. *Mycologia* **50**, 66–79.
- Vitellaro-Zuccarello, L. (1973). Ultrastructure of the byssal apparatus of *Mytilus galloprovincialis*. I. Associated fungal hyphae. *Mar. Biol. (Berlin)* **22**, 225–230.
- Voisin, P. (1931). Biologie ostréicole. La maladie des huîtres de Zélande. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **4**, 221–222.
- Zelezinskaya, L. M. (1972). Investigation of fungus infections of marine Cladocera. *Gidrobiol. Zh.* **8**, 85–87 (in Russian).

5

Protozoans

I. INTRODUCTION

The discussion of protozoan diseases of shellfish—molluscan and crustacean—results in one of the most extensive chapters in this volume, since, as was pointed out by Sparks (1985), “Protozoan parasites are the most common cause of disease in invertebrates.” With a wealth of material to choose from, decisions about inclusion or exclusion of material had to depend on assessment of the extent of damage caused by a particular parasite to shellfish species of economic importance. Application of this rather harsh criterion of worth has kept the chapter to a manageable length, but has resulted in neglect of much interesting material on diseases of noneconomic species. Fortunately, information about disease in noncommercial molluscs can be found in excellent books by Lauckner (1983) and by Sparks (1985) and about diseases in noncommercial crustaceans in the book by Sparks (1985). The original literature on a few diseases, such as ascetosporan (haplosporidan) diseases of oysters and microsporean diseases of shrimp, is surprisingly extensive, even though major gaps in understanding still persist.

To achieve some semblance of order, the principal protozoan diseases of molluscs and crustaceans are considered in turn in this chapter, grouped according to hosts—oysters, mussels, clams, and marine borers—and then shrimp and crabs. To provide some taxonomic reference points, protozoan phyla represented and pathogens considered in the text are shown in Table 6.

II. PROTOZOAN DISEASES OF MOLLUSCS

The best information on molluscan diseases of protozoan etiology has been assembled for oysters, principally because of their great economic

Table 6. Protozoan Pathogens of Molluscs and Crustaceans Considered in This Chapter

Mollusca	Crustacea
Phylum Sarcomastigophora	
<i>Hexamita nelsoni</i> (oysters)	<i>Paramoeba pernicioso</i> (blue crab) <i>Hematodinium perezii</i> (blue crab)
Phylum Apicomplexa	
<i>Perkinsus marinus</i> (oysters)	<i>Nematopsis</i> spp. (shrimp and crabs)
<i>Nematopsis ostrearum</i> (oysters)	
<i>Hyaloklossia pelseneeri</i> (clams)	
<i>Pseudoklossia</i> sp. (clams)	
Phylum Microspora	
<i>Steinhausia mytilovum</i> (mussels)	<i>Ameson nelsoni</i> (penaeid shrimp)
<i>Steinhausia ovicola</i> (oysters)	<i>Thelohania duorara</i> (penaeid shrimp)
	<i>Pleistophora</i> sp. (penaeid shrimp)
	<i>Agmasoma penaei</i> (penaeid shrimp)
	<i>Microsporidium</i> sp. (pandalid shrimp)
	<i>Thelohania butleri</i> (pandalid shrimp)
	<i>Ameson michaelis</i> (blue crab)
	<i>Thelohania maenadis</i> (European shore crab)
	<i>Pleistophora cargoii</i> (blue crab)
Phylum Ascetospora	
<i>Haplosporidium nelsoni</i> (oysters)	<i>Haplosporidium</i> spp. (crabs)
<i>Haplosporidium costale</i> (oysters)	
<i>Haplosporidium tumefaciens</i> (mussels)	
<i>Haplosporidium</i> sp. (shipworms)	
<i>Marteilia refringens</i> (oysters)	
<i>Marteilia sydneyi</i> (oysters)	
<i>Marteilia maurini</i> (mussels)	
<i>Bonamia ostreae</i> (oysters)	
Phylum Ciliophora	
	<i>Paranophrys maggii</i> (European shore crabs)
	<i>Paranophrys</i> sp. (Dungeness crab, American lobster)

importance in many countries. As an example, a variety of Protozoa⁶ parasitize American oysters, *Crassostrea virginica* (Rosenfield, 1964; Sindermann, 1966; Sindermann and Rosenfield, 1967; Sprague, 1970b), and some of them are serious pathogens (Table 7). Two members of the

⁶The former phylum Protozoa has been elevated to the status of subkingdom by committee decision (Levine *et al.*, 1980), and its species have been apportioned among seven phyla: Sarcomastigophora, Labyrinthomorpha, Apicomplexa, Microspora, Ascetospora, Myxozoa, and Ciliophora. Similarly, members of the former class Sporozoa have now been assigned to four phyla: Apicomplexa, Microspora, Ascetospora, and Myxozoa (the last three have spores, but some of the Apicomplexa do not) (Levine, 1970, 1971;

Table 7. Principal Protozoan Pathogens of Oysters

Host	Disease Name	Pathogen	Disease Signs	Effect on Host	Geographic Distribution	References
<i>Crassostrea virginica</i> (American oyster)	Delaware Bay disease, MSX disease	<i>Haplosporidium nelsoni</i> (Ascestospora)	Infected animals thin with mantle recession; disease progressive, becoming systemic; multinucleate plasmodia typical, but sporulation rare	Epizootics and mass mortalities up to 95% occurred in Delaware and lower Chesapeake Bays beginning in late 1950s	Maine to Florida (severe effects from New Jersey to Virginia)	Haskin <i>et al.</i> (1966), Couch <i>et al.</i> (1966), Andrews and Wood (1967), Farley (1968), Andrews and Frieman (1974), Andrews (1977a), Ford (1988)
<i>C. virginica</i>	Seaside disease (SSO)	<i>Haplosporidium costale</i> (Ascestospora)	Infections systemic, with synchronous sporulation in connective tissue	Causes annually summer mortalities with sharp peaks	Seaside bays of Virginia and Maryland	Wood and Andrews (1962), Andrews and Castagna (1978)
<i>Ostrea edulis</i> (European flat oyster)	Digestive gland disease, Aberdeen disease	<i>Marteilia refringens</i> (Ascestospora)	Pale digestive gland, thin meats; focus of infection and sporulation is digestive gland tubules	Epizootics and mass mortalities up to 90% on French Atlantic coast beginning in 1967	France (Atlantic coast), Spain, Holland	Comps (1970), Herrbach (1971), Grizel <i>et al.</i> (1974a,b), Perkins (1976b), Alderman (1979), Grizel (1979)
<i>Saccostrea commercialis</i> (= <i>Crassostrea commercialis</i> ; Sydney rock oyster)		<i>Marteilia sydneyi</i> (Ascestospora)	Infected animals in poor condition, with no gonad development;	Sporadic mass mortalities reported	East coast of Australia	Wolf (1972), Perkins and Wolf (1976)

(continued)

Table 7. (Continued)

Host	Disease Name	Pathogen	Disease Signs	Effect on Host	Geographic Distribution	References
<i>O. edulis</i>	Bonamiasis, hemocyte parasitosis	<i>Bonamia ostreae</i> (Asctospora) (tentative)	massive invasion and destruction of digestive gland tubules Infected oysters may have surface lesions or deep ulcers, tissues have dense granuloocyte invasion, parasites often in hemocyte vacuoles, spores not observed	Epizootics and continuous mass mortalities began in 1979 and spread quickly to all growing areas	Atlantic coast of France, Spain, Holland, Denmark, England	Comps <i>et al.</i> (1980), Pichot <i>et al.</i> (1980), Brehélin <i>et al.</i> (1982), Grizel and Tige (1982), Balouet <i>et al.</i> (1983)
<i>C. virginica</i>	"Dermo" disease	<i>Perkinsus marinus</i> (Apicomplexa) (tentative)	Infected oysters lose weight, infection provokes intense leucocyte invasion of tissues, necrosis of digestive tract epithelium, terminal infections are systemic	Mortalities beginning in the 1940s in the Gulf of Mexico; persistent annual mortalities in high salinity waters	Gulf of Mexico; Atlantic coast of the United States northward to Delaware Bay	Mackin <i>et al.</i> (1950), Mackin (1951), Ray (1952), Mackin and Boswell (1956), Mackin (1962), Mackin and Ray (1966), Andrews (1976b), Perkins (1976a), Perkins and Menzel (1966, 1967)

phylum Ascomycota, *Haplosporidium nelsoni* and *Haplosporidium costale*, and one member of the phylum Apicomplexa, *Perkinsus marinus*, have caused oyster mortalities on the North American East Coast since the 1950s, and all three organisms are still considered serious pathogens.

A. *Haplosporidium nelsoni*

Haplosporidium nelsoni (an ascomycete protozoan commonly referred to as "MSX") has a distribution from Maine to Florida. It has caused extensive mortalities and a drastic decline of the oyster fishery in Delaware Bay beginning about 1957 and in lower Chesapeake Bay beginning in 1959 (Mackin, 1960; Haskin, 1961; Engle and Rosenfield, 1963; Andrews, 1964). In each affected area mortalities exceeded 95% for several years. Because of the severe impact of the *H. nelsoni* epizootic on oyster stocks of the Middle Atlantic states, a number of research groups—university, state, and federal—participated in extensive scientific studies during the late 1950s and the 1960s. Significant papers were published by Haskin *et al.* (1966), naming the plasmodial stage of the parasite as *Minchinia nelsoni* (renamed *Haplosporidium nelsoni* by Sprague, 1978); by Couch *et al.* (1966), associating the plasmodium with spore and prespore stages (Fig. 13); and by Barrow and Taylor (1966), confirming, by immunological techniques, the association of plasmodium and spore. Andrews (1964, 1966, 1968) described aspects of the epizootiology of the disease in Virginia waters, and Haskin *et al.* (1965) briefly summarized the epizootiology in Delaware Bay.

Farley (1965, 1968), after completing a 5-year histopathological study of Chesapeake Bay oysters, categorized natural infections according to the extent of the invasion and the nature of the host response. He found that initial infections occurred in epithelia of gills and palps. The pathogens spread into adjacent connective tissue, provoking infiltration of hyaline hemocytes. In intermediate infections the haplosporidan plasmodia (Fig. 14) invaded connective tissue adjacent to the digestive tract and gonad, and advanced infections were recognized by generalized invasion and infiltration of connective tissue by hyaline hemocytes. Terminal infections were characterized histologically by pyknosis

Levine *et al.*, 1980). To provide some logical reference points for the discussions in this book, the classification by Levine *et al.* will be followed, recognizing that the taxonomy of parasitic Protozoa is in a "dynamic" state, with frequent changes in specific and generic names of familiar and not-so-familiar parasites. Most of the severe protozoan pathogens of molluscs have been assigned to the newly described phyla Ascomycota or Apicomplexa (see Table 7 for examples).

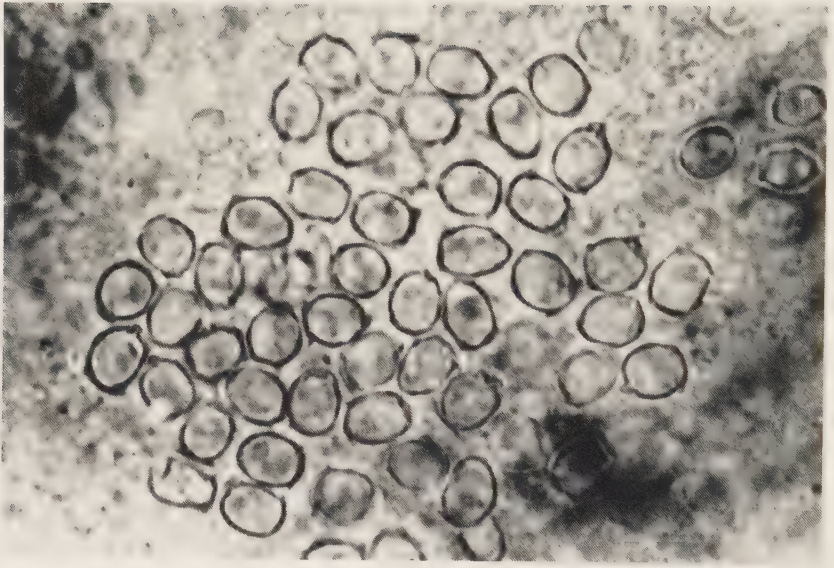


Figure 13. Spores of the haplosporidan oyster pathogen *Haplosporidium nelsoni*.

of nuclei and tissue necrosis. Farley also recognized a stage of the disease termed "remission," identified by diminished intensity of infection and cell infiltration, localization of parasites near external epithelia, increased pigment cell formation, diapedesis, deposition of necrotic tissue and moribund parasites against the shell, and conchiolinous encapsulation (Fig. 15). Death from *H. nelsoni* infection was attributed to the combined action of seasonal environmental or physiological stresses on oysters weakened by effects of the disease.

Farley (1967) also proposed a detailed life cycle of *H. nelsoni* in the oyster, based on a study of over 1000 natural infections over a 5-year period. Multinucleate plasmodia develop from uninucleate infective stages, then reproduce by plasmotomy. Gametic nuclei fuse within large plasmodia and undergo sporogonic divisions. Sporoblasts differentiate into spores in the epithelium of digestive diverticula. Seasonal variations in occurrence of particular life cycle stages were noted: initial infections were most common from April to September, parasite activity decreased during the colder months (December through March), and sporogony occurred in midsummer.

The effects of *H. nelsoni* on the U.S. oyster fishery have been clearly demonstrated, especially in Delaware Bay. Landings in New Jersey waters of Delaware Bay in the late 1940s and early 1950s had fluctuated around 6 million pounds of shucked meats. Disease decimated the

stocks, beginning in the mid-1950s. Landings fell precipitously to a low of 167,000 pounds in 1960 and have not recovered significantly. Recently, in a report based on more than two decades of continuous sampling (a remarkable achievement in marine pathology), Ford and Haskin (1982) estimated that at least half of all oyster deaths in lower Delaware Bay since the early 1960s could be attributed to the effects of *H. nelsoni* infections—a staggering figure indeed!

Comparable effects were felt in the high-salinity waters of lower Chesapeake Bay, another major oyster-producing area (Andrews, 1967a). *H. nelsoni* occurs in waters whose summer salinity consistently exceeds 15 ‰. During years of drought (1963–1965) along the Atlantic coast, the pathogen invaded areas of middle Chesapeake Bay formerly free of the disease (Rosenfield and Sindermann, 1966). Observational evidence for the decisive role of salinity in the epizootiology of the disease was presented by Andrews (1964). Simultaneous examinations of salinity regimens, disease prevalence, and mortalities in the James River seed beds of Virginia disclosed that infections disappeared from areas of marginal disease occurrence during seasons of low salinity and during several years of high rainfall. The importation of infective agents from high-salinity areas of the lower estuary of the James River was postulated to explain the persistence of the disease in marginal-salinity zones.

A detailed examination of the relationship of *H. nelsoni* and salinity in Delaware Bay was made by Haskin and Ford (1982). Based on more than 20 years of sampling along a salinity gradient in that estuary, they concluded:

1. Prevalence of the disease showed a regular decrease from high to low salinity.
2. Intensity of infection and disease-related mortalities showed a sharp drop between grow-out areas in high salinities and seed-producing areas in low salinities; annual mortalities averaged 30% during the first year after transfer to the grow-out areas but only 4–9% on the seed beds.
3. The discontinuity in infection intensity and mortality rates suggested a salinity threshold which had little effect on distribution or infectivity of the pathogen but a pronounced effect on the course of the disease once infection was achieved. Based on their extensive observations, Haskin and Ford concluded that the parasite does not survive in salinities below 10 ‰; between 10 and 20 ‰ infections are acquired in a pattern that is directly correlated with salinity, but the development of infections and mortality levels are reduced; full parasite activity occurs above 20 ‰, but may decrease above 30 ‰.

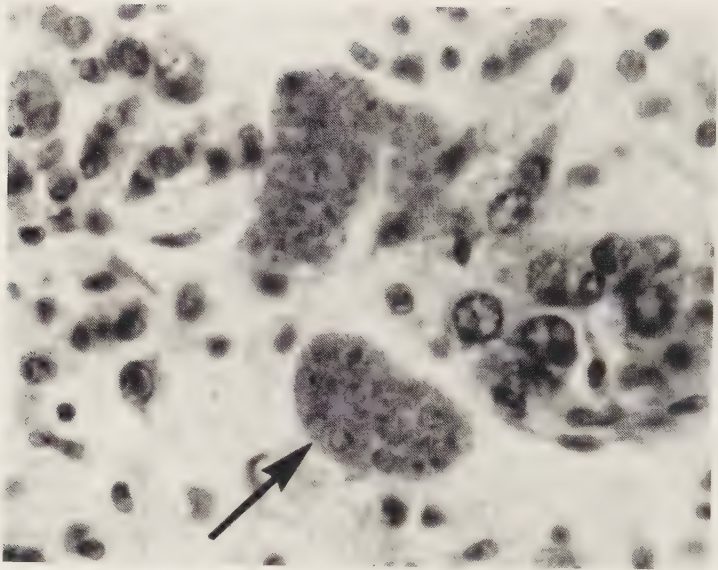


Figure 14. Plasmodia of *Haplosporidium nelsoni* in oyster tissue.

Experimental evidence for negative effects of low salinity on the pathogen was also reported by Sprague *et al.* (1969). Infected oysters were placed in tanks of different salinities for 6 months. Upon examination, infections had disappeared in oysters held at 7–8 ‰, whereas prevalence in oysters held at 19–22 ‰ was 80%. Similar results were reported by Haskin and Ford (1982).

Temperature is also an important factor in the epizootiology of *H. nelsoni*. Andrews (1966) found that in Virginia oysters infections persisted through the winter; death rates decreased with decreasing temperature in autumn but rose again in February and March. Infection of new hosts and mortality peaks occurred primarily during the warm season.

Thus, seasonal temperature changes are clearly of great significance to the annual cycle of *H. nelsoni*. High summer temperatures enhance activity of the pathogen, but also the internal defense mechanisms of the host. Based on field and laboratory observations, it appears that below 5°C both parasite and host are inactive; between 5 and 20°C the parasite multiplies faster than the host can contain it; above 20°C resistant oysters can inhibit parasite multiplication or eliminate it from tissues (Ford and Haskin, 1982).

Proximity of oysters does not seem to be a significant epizootiological factor in Delaware Bay disease. Andrews (1966, 1967b) found continued high levels of disease activity in lower Chesapeake Bay, even after oyster populations had been drastically reduced. This phenomenon suggests strongly that a reservoir host exists, although surveys have thus far failed to disclose it.

With more than 25 years of observation of the disease in Delaware Bay, Haskin and associates have discerned some evidence for a 6- to 8-year cycle of activity, with peaks in 1957–1959, 1964–1967, 1972–1976, and 1979–1980 (Ford and Haskin, 1982; Haskin and Ford, 1983). Low disease activity was associated in several instances with unusually low winter temperatures in the preceding year, whereas high prevalences occurred during periods of moderate or high winter temperatures—suggesting a role for low temperature in determining disease effects at that latitude. Despite these apparent relationships, however, Ford and Haskin (1982) postulated that cyclic pathogen levels in oysters may really reflect cyclic abundance of an as-yet-undetermined reservoir host [one possible candidate is the shipworm, *Teredo navalis*, which was found by Hillman (1978, 1979) and by Hillman *et al.* (1982) to be infected by an organism with spores which were very similar to those of *H. nelsoni* from oysters]. Temperature could still be significant, of course, acting indirectly through regulation of reservoir host population abundance.

The disease caused by *H. nelsoni* has continued to be a problem for the oyster industry of the Middle Atlantic states to the present time. Recurrent outbreaks with accompanying mortalities have occurred in Delaware Bay and Chesapeake Bay although total mortalities have been less than those of the late 1950s and the 1960s (except in the Maryland portion of Chesapeake Bay, where the disease is now epizootic). Excellent summaries of new information and disease status have been published (Andrews and Frierman, 1974; Farley, 1975; Andrews, 1979; Ford and Haskin, 1982, 1987; Haskin and Ford, 1979, 1982, 1983, 1987; Ford, 1985a,b, 1986, 1988; Ford and Figueras, 1988). Some of the most interesting information concerns the apparent development of resistance to the pathogen in oyster populations exposed continuously to infection. Evidence for increased resistance of native Delaware Bay stocks was summarized by Haskin (1976), Haskin and Ford (1979), Ford and Haskin (1982, 1987), and Ford (1988) as follows:

1. A progressive yearly decline was seen in the proportion of systemic infections compared to total infections, dropping from 76–80% of infected oysters in 1958–1964 to only 35–58% in



Figure 15. Blisters on the inner shell of *Crassostrea virginica*, which are actually conchiolinous encapsulations of necrotic phagocytic cells and the pathogen *Haplosporidium nelsoni*.

1972–1975. This change indicated that more infected oysters were able to contain the pathogen in localized nonlethal lesions (Ford and Haskin, 1982).

2. A reduction in mortalities was observed among second- and third-generation offspring of native stocks. Third-generation native oysters exposed continuously to infection had a cumulative 3-year mortality of only 34–42%, compared with a 92% cumulative mortality for introduced susceptible stocks (Haskin, 1976).
3. Surviving oysters in epizootic areas undergoing second or third annual infection periods had lower mortality rates than oysters exposed to the pathogen for the first time (Ford and Haskin, 1982).
4. Laboratory-reared progeny of oysters that survived several generations of selection for disease resistance had lower mortality rates than unselected imports (Haskin and Ford, 1979).
5. Resistant oysters seem able to restrict infections to localized nonlethal levels and to tolerate—often for prolonged periods—parasite burdens that would kill susceptible oysters (Ford, 1985a; Ford and Haskin, 1987).

Findings consistent with the above have been reported for Chesapeake Bay oysters. Native populations, after 3 years of exposure, had more light infections with inactive parasites, while introduced populations had more heavy infections with rapidly proliferating parasites and intense hemocytic response (Farley, 1975). In high-salinity waters near the Bay mouth, oysters that had been selected by exposure in trays for several generations did not usually show patent infections, and mortalities in trays were reduced to 10% annually (Andrews, 1979).

B. *Haplosporidium costale*

A second ascetosporan protozoan from oysters, *C. virginica*, on the Atlantic coast of North America is *Haplosporidium costale* (sometimes referred to as SSO, or seaside organism). It has a more restricted distribution, exerting its effects in the high-salinity seaside bays of Maryland and Virginia, along the lower eastern shore of Virginia, and to a lesser extent in Delaware Bay (Andrews *et al.*, 1962; Wood and Andrews, 1962; Sprague, 1963b; Haskin *et al.*, 1966; Couch, 1967b). First recognized in moribund and dead oysters from Hog Island Bay, Virginia, by Wood and Andrews (1962), *H. costale* was held responsible, on the basis of epizootiological evidence, for sharp peaks of mortality in May and June. Striking characteristics of the disease are the sudden onset of mortalities in May and the abrupt termination of mortalities about mid-June. The

Table 8. Distinguishing Characteristics of the Oyster Pathogens *Haplosporidium nelsoni* and *Haplosporidium costale*^a

<i>Haplosporidium nelsoni</i>	<i>Haplosporidium costale</i>
Occurs in 15–25‰ salinity waters of United States East Coast from Maine to Florida	Occurs in seaside bays of Delaware, Maryland, and Virginia and the bay-side of Virginia's eastern shore in salinities >25‰
Initial infection site is usually the gill epithelium	Initial infection site is digestive gland tubule epithelium
Sporulates rarely in epithelium of host digestive gland tubules	Sporulates commonly in host connective tissue
Spores average 8 μ m and life history stages are larger than those of <i>H. costale</i>	Spores average 4 μ m
Most plasmodia remain in that stage, even when rare sporulation occurs in a given host	Almost all plasmodia sporulate
Localized (chronic) infections seen commonly in resistant hosts	Localized infections seen only rarely
Infective period June–October	Infective period June–July
Mortalities occur 1–10 months after exposure; highest mortalities occur in late summer and autumn, but may occur year-round	Mortalities occur 10 months after exposure to the pathogen—only as a sharp peak in May–June
Mortalities of susceptible stocks in enzootic areas average 40–70%	Mortalities of native stocks in restricted enzootic area average 20–50%

^a After Andrews and Castagna (1978) and Andrews (1984).

pathogen and mortalities have persisted in Maryland and Virginia sea-side oyster populations (Couch, 1967b; Couch and Rosenfield, 1968; Andrews and Castagna, 1978).

Haplosporidium costale, unlike *H. nelsoni* (Table 8), sporulates synchronously in host connective tissues in early summer, after a 2- to 3-month developmental period, but is not seen at other times. Mortalities due to the disease reach a sharp peak in June, then decline just as sharply (Andrews, 1976a, 1977b; Andrews and Castagna, 1978). Development of the pathogen is enhanced in salinities greater than 30 ‰, and there is some evidence for regression of plasmodial infections in some years. An 18-year study (1959–1977) of *H. costale* in Virginia waters, summarized by Andrews and Castagna (1978), concluded that the disease caused consistent annual mortalities of 50% or higher in some sampling areas.

Although several life history stages have been recognized for both North American species of *Haplosporidium*—*H. nelsoni* and *H. costale*—and concurrent infections have been described (Couch, 1967b), routes of infection and methods of transmission are still unknown, and Koch's postulates have not been fully satisfied. Studies have been further hampered by the inability to maintain infections in oysters under experimental conditions for extended periods. Because several research groups are actively concerned with oyster diseases on the U.S. East Coast, particularly with *H. nelsoni*, increased understanding of this and other pathogens has developed during the past several decades. Andrews (1968) presented a thought-provoking review of evidence and hypotheses concerned with the epizootiology of *H. nelsoni* and presented findings indicating that seed oysters could be successfully planted in epizootic areas, if this were done at a very early age. Couch and Rosenfield (1968) summarized a well-conceived and -executed study of the fate of disease-free oysters introduced into an epizootic area. Ford and Haskin (1982) and Andrews (1984) have provided up-to-date reviews of current thinking about the epizootiology of haplosporidan diseases of oysters from U.S. waters.⁷

C. *Perkinsus marinus*

A third protozoan pathogen from the North American East Coast, recently placed in the phylum Apicomplexa (Levine, 1978), is *Perkinsus marinus*. This taxonomically problematic protistan, particularly common in Gulf of Mexico and lower Chesapeake Bay oyster populations, was

⁷This seems to be a logical place to point out that several *Haplosporidium* spp. organisms have been reported as rare parasites of other species of oysters. The European flat oyster, *Ostrea edulis*, for example, may be infected by *Haplosporidium armoricanum* (originally described as *Minchinia armoricana*). The parasite was reported from flat oysters imported to The Netherlands from France (van Banning, 1977, 1979) and was later found on the Brittany coast of France (Cahour *et al.*, 1980). Other European reports of *Haplosporidium* spp. in *O. edulis* have appeared, but the precise taxonomy has not been resolved (Pichot *et al.*, 1979; Bachere and Grizel, 1982; Vivares *et al.*, 1982; Pichot 1986). Observations of significance were that prevalences were very low and that none of the organisms was recognized as a severe pathogen. *Haplosporidium*-like parasites have also been reported from another species of the genus *Ostrea*, *Ostrea lurida*, from Oregon (Mix and Sprague, 1974). Prevalences ranged from 0 to 5%, only plasmodial stages were seen, and no mortalities were associated with the occurrence of the parasite. Pacific oysters, *Crassostrea gigas*, also harbor *Haplosporidium*-like organisms. Kern (1976) found sporulating organisms very similar to *H. nelsoni* in samples from Korea (in four of 1438 oysters), and Katkansky and Warner (1970) found a sporulating *Haplosporidium* sp. in a single specimen of *C. gigas* from California. In neither case were extensive or localized mortalities associated with presence of the parasites.

first described by Mackin *et al.* (1950) as a fungus, *Dermocystidium marinum*, and later as *Labyrinthomyxa marina* by Mackin and Ray (1966). The pathogen has been the subject of much research since it was first recognized by Mackin *et al.* as a major cause of oyster mortalities in the Gulf of Mexico. Diagnosis of infection was aided by early development of a technique based on a fluid thioglycollate medium fortified with an antibiotic (Ray, 1952, 1966b). A number of authors have shown that *P. marinus* causes recurrent oyster mortalities (Mackin *et al.*, 1950; Mackin, 1953; Ray, 1954a–c; Andrews and Hewatt, 1954; Hewatt and Andrews, 1954; Kern *et al.*, 1973; Andrews, 1979). Pathological changes in infected oysters were described by Mackin (1951). Invasion takes place through the gut epithelium and possibly through the mantle. The epithelium is destroyed; the parasite lyses the basement membrane and is distributed by the blood to all parts of the body (Fig. 16). All tissues are invaded and damaged, and multiple abscesses are formed. Normal gonadal development is inhibited, infected oysters become severely emaciated (Ray *et al.*, 1953; Ray 1954b), and growth is retarded (Menzel and Hopkins, 1955).

Temperature is important in the epizootiology of *P. marinus* disease (Hewatt and Andrews, 1956; Quick and Mackin, 1971). Infections and associated mortalities rise during the warm months and decline during

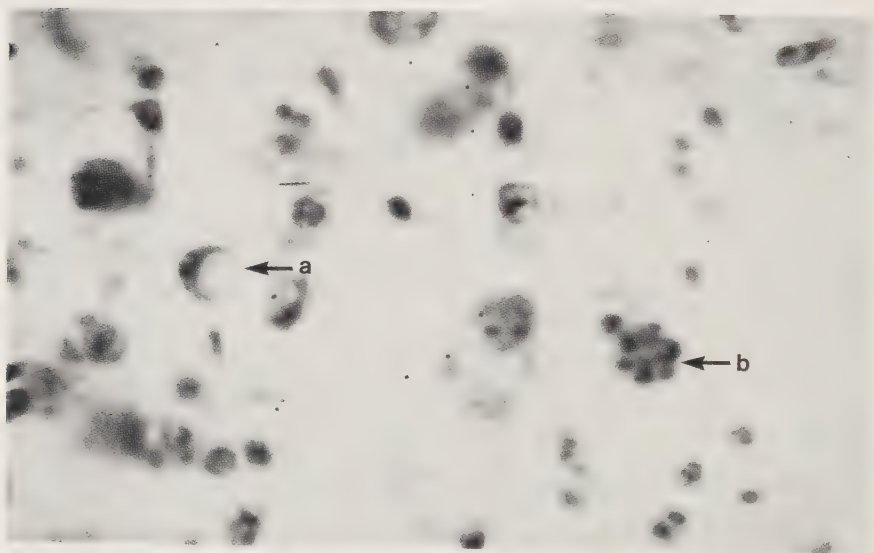


Figure 16. Characteristic life cycle stages of the protistan *Perkinsus marinus*. (a) Pre-zoosporangia ("hypnospores" of the earlier literature) and (b) daughter cells formed by cleavage of protoplast ("rosette" of the earlier literature).

colder periods. Mortalities decline in winter, probably because of reduced parasite metabolism, rather than elimination of the organism. Failure to find the pathogen consistently north of Chesapeake Bay suggests that prolonged low temperature may be a significant limiting factor. Andrews (1965, 1967b) found that the parasite proliferated readily only at temperatures above 25°C and overwintered as subpatent infections. He further observed that partial destruction of lower Chesapeake Bay oyster populations by another protozoan disease (caused by *H. nelsoni*) resulted in decreased prevalence of *P. marinus*, suggesting that *P. marinus* depends on direct transmission from one oyster to another. Other evidence for direct transmission was provided by Ray and Mackin (1955), Mackin (1951, 1961), and Andrews (1979). However, alternate routes of infection may exist. Hoese (1964) was able to find the organism in the digestive tracts and feces of fish, oyster drills, and crabs that had fed on dying and dead infected oysters. He speculated that transmission might be furthered by scavengers and that scavengers may release the parasite from host tissue. An additional route of infection has been demonstrated recently; the ectoparasitic pyramidellid snail, *Boonea impressa*, has been shown to be capable of transmitting *P. marinus* from infected to uninfected oysters (White *et al.*, 1987).

Infections and resulting mortalities are reduced in salinities below 15 ‰. Ray (1954b) showed that low salinity retarded development of terminal infections in laboratory populations. Ray and Chandler (1955) suggested that excessively high salinities may also be unfavorable for *P. marinus*. Mackin (1956) found a positive correlation between high salinity and high incidence of the fungus, but observed in experimental studies that the salinity tolerance range was wide. Dilution of the infective elements by inflow of fresh water was suggested as an important limiting factor and a possible control measure.

Perkinsus marinus is abundant in waters of the southern United States. Ray (1966a), who surveyed the occurrence of the pathogen in the Gulf of Mexico in 1961 and 1962, found infections in 35 of 39 oyster samples, and prevalences as high as 100%. The pathogen has persisted at near-epizootic levels in many oyster-producing areas of the Gulf of Mexico, where losses in excess of 50% have occurred annually in some beds (Quick and Mackin, 1971; Little and Quick, 1976; Andrews, 1979; Ogle and Flurry, 1980). However, the disease is best represented as one causing continuing attrition, with periodic outbreaks when environmental conditions and host population density permit.

Perkinsus marinus is common in oysters from high-salinity areas of the South Atlantic and Gulf of Mexico coasts of the United States, but is absent in a few such areas. Hoese (1963) attempted assays of water

samples from different locations in one section of the Texas coast, to determine whether seawater from some areas inhibited development. Although results were not conclusive, water samples from certain localities apparently did inhibit development. Hoese speculated that the absence of *P. marinus* may be related to the presence of *Spartina* salt marsh where consistently high salinities occur. It should be noted, however, that *Spartina* also predominates in some low-salinity, high-*Perkinsus* areas.

In addition to its common occurrence in *C. virginica*, *P. marinus* has been found in other species. Ray (1954b) reported it in the leafy oyster, *Ostrea frons*, from Florida and in horse oysters, *Ostrea equestris*, from Texas. The organism was *not* found, however, in mangrove oysters, *Crassostrea rhizophorae* from Puerto Rico, in *Ostrea edulis* from Holland, or in the rock oyster, *Saccostrea commercialis*, from Australia. The Olympia oyster, *Ostrea lurida*, was experimentally infected by exposure to infected *C. virginica*. *Perkinsus*-like organisms have also been seen in other molluscs and annelids. Andrews (1955) found what he termed "*Dermocystidium*-like" organisms in 12 of 16 mollusc species from the Chesapeake Bay area (*Dermocystidium*, you will recall, is the earlier name for *Perkinsus*).

This important pathogen of oysters has been the subject of continuing research for more than 30 years. Knowledge of its biology was summarized by Ray (1954b), Ray and Chandler (1955), Andrews and Hewatt (1957), and Mackin (1961). Mackin and Boswell (1956) proposed a life cycle for *P. marinus* that included a saprophytic stage leading to the production of an infective spore. Perkins and Menzel (1966) described motile biflagellate zoospore stages that were also postulated to be infective to oysters. In a later study using electron microscopy, Perkins (1969, 1976a) observed that the zoospore contained a so-called "apical complex" characteristic of members of the phylum Apicomplexa. Based on this and other features, Levine (1978) renamed the pathogen *Perkinsus marinus* and transferred it to the Apicomplexa.

Oysters from European waters have had problems with protozoan pathogens that parallel those in North America. Within the past two decades European flat oyster (*O. edulis*) populations have been seriously affected by two ascetosporean parasites—*Marteilia refringens* and *Bonamia ostreae*—which are considered next.

D. *Marteilia refringens*

The ascetosporean *Marteilia refringens* was first observed in dying oysters (*O. edulis*) on the Atlantic coast of France in 1968 and was first reported

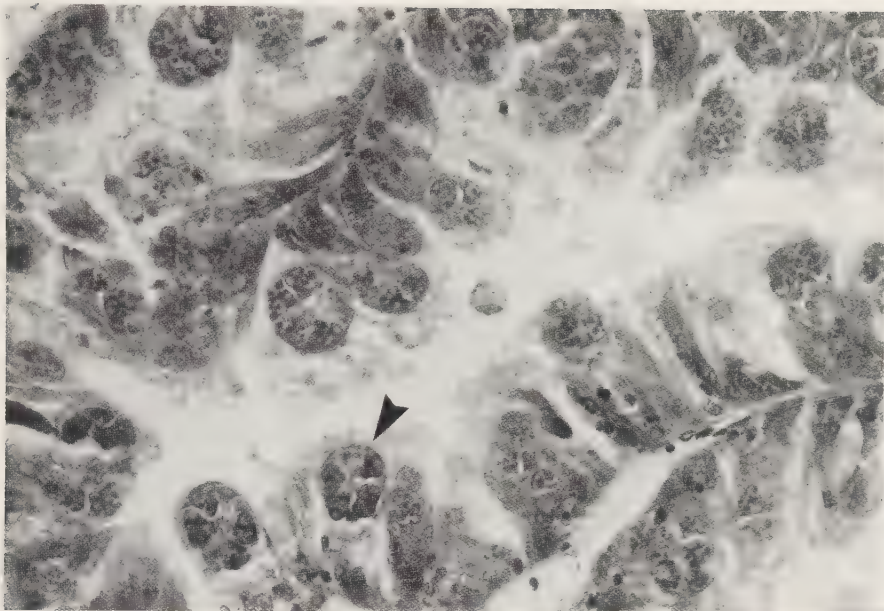


Figure 17. Stages in the life history of *Marteilia refringens*. (Courtesy of Mr. C. A. Farley.)

in the scientific literature by Comps (1970). Despite some persistent questions about its affinities, the pathogen was named by Grizel *et al.* (1974a). It exerts its most serious pathological effects on the intestine and digestive gland tubules—hence the common name “digestive gland disease.” A complex life cycle was described by Grizel *et al.* (1974b) and by Grizel and Tige (1979) involving plasmodial and sporulating stages (Fig. 17), and was further elaborated by Balouet (1979) and by Franc (1980). A recently developed method to isolate and purify sporangia of *Marteilia* should improve diagnostic techniques and should contribute to knowledge of its life cycle and physiology (Mialhe *et al.*, 1985).

The disease progresses slowly, with peak mortalities in summer, 1 year after the oysters are planted, and with significant (up to 40%) weight loss prior to the time of death (Morel and Tige, 1974). Prevalences of up to 100% have been reported, with extreme variability from one growing area to another. The parasite has also been seen in low prevalences in introduced Pacific oysters, *Crassostrea gigas*, on the French coast (Balouet *et al.*, 1979; Cahour, 1979), and in mussels, *Mytilus edulis*, from France and Spain (Comps *et al.*, 1975; Gutiérrez, 1977a,b). *Marteilia*

refringens has been introduced into Spanish and Dutch waters with imports of flat oysters of French origin, but severe effects in those countries have not been demonstrated conclusively (Grizel, 1976; Masso-Bolivar, 1978; van Banning, 1979).

E. *Bonamia ostreae*

A second important protozoan pathogen of European flat oysters, *O. edulis*, is the presumptive ascetosporan *Bonamia ostreae*. The disease that it causes (bonamiasis) and related mortalities were first observed on the Brittany coast of France in 1979 (Comps *et al.*, 1980; Pichot *et al.*, 1980; Tige *et al.*, 1980; Grizel, 1985). The disease is characterized by yellow discoloration of the oyster, extensive gill lesions, the presence of so-called "microcells" (Fig. 18), and a massive host cellular response. Hemocytes are the principal focus of infection; necrosis of the digestive epithelium may also occur. Plasmodial stages of the organism have been described, and ultrastructural characteristics (particularly the presence of "haplosporosomes") suggest affinities with the haplosporidans (as-

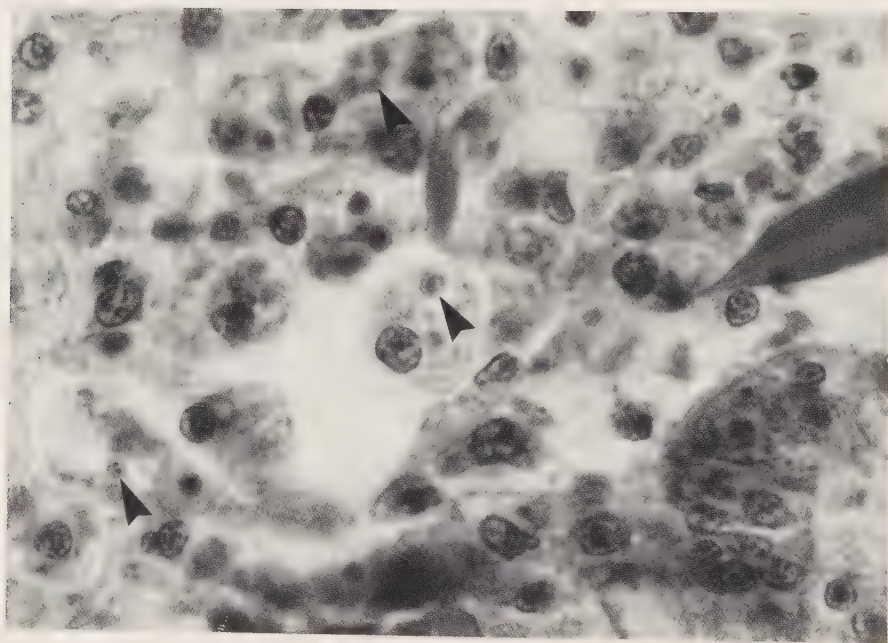


Figure 18. *Bonamia ostreae* in tissues of the European flat oyster, *Ostrea edulis*. (Courtesy of Mr. C. A. Farley.)

cetosporens), even though spore stages have not been reported (Brehélin *et al.*, 1982; Comps, 1982).

From its first observation in Brittany in 1979, the disease spread to other French oyster-producing areas, and was imported into The Netherlands with infected oysters (Grizel and Tige, 1982; Balouet *et al.*, 1983). Mortalities in some French growing areas reached 80%, with active infections seen year-round and lethal infections developing within 6 months after introduction of susceptible oysters (Poder *et al.*, 1982). A ban on importation of seed oysters from Brittany into The Netherlands and an extensive program to remove all populations of flat oysters from infected areas have limited the disease in that country (van Banning, 1982, 1985). Bonamiasis and associated mortalities have also been reported from Spain, Denmark, the United Kingdom, and the United States (Bucke *et al.*, 1984; Bucke and Feist, 1985; Elston *et al.*, 1986). A hypothesis has been proposed that the epizootic on the French coast may have been the consequence of importation of infected seed oysters from a California estuary (Elston *et al.*, 1986), although the earlier history of introduced *O. edulis* in U.S. waters is murky, and alternate explanations for the recent outbreak in France seem equally plausible.

A *Bonamia* sp., with characteristics slightly different from those of *B. ostreae*, has been reported recently to cause a lethal hemocytic disease of New Zealand dredge oysters, *Tiostrea lutaria* (Dinamani *et al.*, 1987). Mortalities of 63% were seen in some wild populations in 1986. A seasonal pattern of presumed developmental stages was described, including a possible infective stage.

F. *Marteilia sydneyi*

Protozoan pathogens of oysters have been reported from other geographic areas, in addition to North America and Europe. Mass mortalities of Sydney rock oysters (*Saccostrea commercialis*) from eastern Australia have been attributed to epizootics of the ascetosporean *Marteilia sydneyi* (Wolf, 1972; Perkins and Wolf, 1976). The disease is characterized by massive invasion and necrosis of digestive gland epithelium; infected oysters are remarkably thin; and gonadal development is inhibited. Death occurs within 2 months after infection, and mortalities were reported sporadically for 7 years (1971–1978), during which estimated losses reached 80% of existing stocks (Wolf, 1979).

G. Other Oyster Parasites of Uncertain or Relatively Minor Significance

In addition to the pathogenic ascetosporens and apicomplexans already considered, a number of protozoans have been considered by some

authors to be pathogens, but by others to be harmless. A classical example is the gregarine *Nematopsis ostrearum*, an apicomplexan claimed by Prytherch (1938, 1940) to be the cause of extensive mortalities in Virginia and Louisiana. Later studies (Sprague, 1949; Sprague and Orr, 1955) indicated, however, that *Nematopsis* did not cause deaths of oysters and suggested that *Perkinsus* infections may have complicated earlier results. Owen *et al.* (1952) found no correlation between *Nematopsis* infections and oyster mortalities in Louisiana. Sprague and Orr (1953, 1955) demonstrated that *N. ostrearum*, as described by Prytherch, was actually two species, which they designated *N. ostrearum* (emended) and *N. prytherchi*. Spores of *N. prytherchi* are larger and more elongated, localize in the gills rather than in the mantle, and have reproductive stages only in the crab *Menippe mercenaria*. The life cycle of *N. ostrearum* includes a mud crab host, *Panopeus herbstii*, *Eurypanopeus depressus*, or *Eurytium limosum*. In many growing areas most oysters are infected, although infections are rarely heavy. Mackin (1962) pointed out the lack of tissue reaction to the parasite and the lack of evidence for the existence of lysins or toxins. Feng (1958) found that a dynamic equilibrium existed between the acquisition by the oyster of new parasites and the elimination of spores. Oysters eliminated *Nematopsis* infections rapidly in the absence of crabs and continued reinfection.

Nematopsis was shown by Landau and Galtsoff (1951) to be widely distributed on the Atlantic and Gulf coasts of the United States. Infections were heavy in oysters from certain localities, such as the mouth of the Chesapeake Bay, but relationships with other ecological factors, such as abundance of crabs, were not apparent. The intensity of infection was cumulative and increased with age of the host. No evidence was obtained to indicate that *Nematopsis* infection killed oysters or reduced the quality of the meats.

While the weight of evidence seems to indicate a lack of lethality of *Nematopsis* infections, their possible pathogenicity is not easily dismissed. Sprague and Orr (1955) pointed out that heavy infections by *N. prytherchi* can clog gill blood vessels. I have observed massive infections by *N. ostrearum*, in which extensive areas of the oyster were occupied by spores. Such mechanical interference with host physiology must have some harmful effects.

A flagellate protozoan, a member of the phylum Sarcomastigophora, redescribed as *Hexamita nelsoni* by Schlicht and Mackin (1968), occurs frequently in the digestive tracts of oysters, but its parasitic or saprozoic role has not been adequately determined. *Hexmita* can serve as another example of disputed pathogenicity. First described by Certes (1882) as a commensal in European oysters, the flagellate was later held

responsible for oyster mortalities from so-called "pit disease" in Holland (Mackin *et al.*, 1952). Clear evidence of pathological effects was not obtained, and heavy bacterial infections complicated the study. *Hexamita* was also blamed for mortalities of Olympia oysters, *Ostrea lurida*, in Washington (Stein *et al.*, 1961), but again clear evidence of pathogenicity was not presented. Scheltema (1962), who examined the relationship between *Hexamita* and American oysters from Delaware Bay, concluded that the organism did not contribute significantly to deaths of oysters. He suggested, as did Stein *et al.* (1961), that *Hexamita* may act as a pathogen during periods of low environmental temperatures, because the processes in oysters which act to remove the trophozoites do not exceed the reproductive rate of the flagellate.

An excellent experimental study of hexamitiasis in oysters was published by Feng and Stauber (1968). Their results showed that survival of the infected host was influenced by temperature, and that the course of infection could be altered by the physiological condition of the host, the size of the inoculum, and the route of entry. The authors believed that further studies were still needed to answer the question of whether *Hexamita* is a true pathogen, since the invasive power of the organism had not been conclusively demonstrated and the role of associated bacteria had not been elucidated.

So, here, then, are the principal protozoan pathogens of oysters—*Haplosporidium nelsoni*, *H. costale*, *Perkinsus marinus*, *Marteilia refringens*, *M. sydneyi*, and *Bonamia ostreae*—as well as some of doubtful pathogenicity (*Nematopsis ostrearum* and *Hexamita nelsoni*).

H. Diseases of Molluscs Other Than Oysters

Probably because of the overriding economic importance of oysters, their diseases—especially those of protozoan etiology which lead to mass mortalities—have received much of the attention thus far in this chapter and in the published literature as well. Some small attempt at balance should be made, however, by pointing out that other molluscs, such as mussels, clams, shipworms, and abalones, can be affected by an array of protozoan pathogens. Effects of protozoan diseases on other molluscs seem pale by comparison to those of oysters, probably due in large part to the much smaller economic importance of those hosts, and the correspondingly smaller research attention paid to their diseases.

Sea mussels, *Mytilus edulis*, may be parasitized by protozoans. Eggs of mussels from the western North Atlantic may be infected with *Steinhausia* (formerly *Haplosporidium*) *mytilovum*, a genus now assigned to the protozoan phylum Microspora. The parasite had a high prevalence in

some samples, although the proportion of infected eggs to normal eggs was low in any single host (Field, 1923; Sprague, 1965b). What seems to be the same organism was recognized in eggs of the Mediterranean edible mussel, *Mytilus galloprovincialis*, from the Gulf of Naples (DeVincentiis and Renzoni, 1963). Another microsporean egg parasite, *Steinhausia* (formerly *Chytridiopsis*) *ovicola*, was described in European flat oysters taken at Marennes, France (Léger and Hollande, 1917). The parasite was rare and occurred only in certain ovarian follicles of affected oysters.

The ascetosporan (haplosporidan) *Haplosporidium tumefacientis* was described as the cause of a disease of California mussels, *Mytilus californianus* (Taylor, 1966). The disease, characterized by tumefactions of the digestive gland and the kidneys, was found in 23 of 1114 individuals examined. Gross enlargement of the organs was apparently due to plasmodia of the parasite; no necrosis was reported. Samples of sea mussels collected simultaneously from the California coast by Taylor were not parasitized by the organism. Operculate spores typical of the group occurred in sporocysts within the subepithelial tissues. Spores survived for 3 months outside the host, but transmission experiments using freshly prepared infected tissues were unsuccessful. Mussels from the lower intertidal zone were more heavily parasitized than those higher in the zone, but mortalities were not reported. Another ascetosporan, *Marteilia maurini*, similar to the flat oyster pathogen, *M. refringens*, has been reported in mussels (*M. galloprovincialis*) from the Lagoon of Venice (Comps *et al.*, 1982). Prevalences in some samples reached 40%, but pathogenicity was not determined.

Still another ascetosporan, an unidentified haplosporidan, was found to infect gaper clams, *Tresus capax*, from the Pacific coast of the United States (Armstrong and Armstrong, 1974). The disease was characterized by macroscopically visible white cysts in the mantle and the siphons. Heavily infected individuals were dark and sluggish, gonadal development was inhibited, and the disease was considered lethal. Prevalences averaged 43% in the study by Armstrong and Armstrong, no spore stages were seen, and host hemocyte response was extensive.

Clams of a number of genera have been reported to be parasitized by coccidians (phylum Apicomplexa). Infections usually occur in the kidney, resulting in extensive tissue destruction and impaired function. The earliest report described *Hyaloklossia pelseneeri* from *Donax* sp. and *Tellina* sp. from the French coast (Léger, 1897), and the most recent described a coccidian with characteristics of the genus *Pseudoklossia* from the little neck clam, *Protothaca staminea*, from the Pacific coast of the United States (Morado *et al.*, 1984). Pathology may be extensive in heavy infections, but even heavy infections do not seem to cause death (Sparks, 1985).

Clams may also be infected by flagellates. An *Isonema* sp. was recently reported as a facultative pathogen of larval geoduck clams, *Panope abrupta*, in a Washington hatchery (Kent *et al.*, 1987). The flagellates penetrated the mantle and proliferated in the coelom; heavy infections caused disintegration of tissues and were lethal to the clam larvae.

Shipworms (Teredinae) may be parasitized by Asctospora. Three species of these bivalve marine borers—*Teredo navalis*, *T. bartschi*, and *T. furcifera*—from the Middle Atlantic coast of the United States were found to be infected with a *Haplosporidium* sp. very similar in size and morphology to *H. nelsoni* from oysters. Prevalences of 9–40% were reported, and pathology was extensive. Gill lesions as well as hemocyte infiltration of connective tissue characterized the infections, and the disease was suggested to be an important regulator of population size (Hillman, 1978, 1979, 1980; Hillman *et al.*, 1982). Early speculations that this organism in borers may actually be *H. nelsoni* have not been substantiated, although the search for an alternate host in the life cycle of the oyster pathogen is continuing.

A protozoan (apicomplexan) disease of the abalone, *Haliotis ruber*, from South Australia was recognized recently (Lester and Davis, 1981). Characterized by yellowish pustules in the musculature of the foot and mantle and high prevalences in some fishing areas, the pathogen was described as *Perkinsus olseni*, with similarities to *P. marinus* from American oysters. Foci of infections in abalone were muscle and hemolymph; experimental transmission to oysters (*Saccostrea commercialis*) was unsuccessful. Another species of *Perkinsus* was suggested as a possible cause of widespread mortalities of *Haliotis laevis* in South Australia (Lester, 1986). Additionally, a recent survey of bivalve molluscs from the Great Barrier Reef of Australia disclosed *Perkinsus* spp. infections in 30 of 84 species examined; heavy infections were noted in some of the samples, including the giant clams, *Tridacna* spp., which had been suffering severe mortalities, with losses of up to one third of some reef populations (Alder *et al.*, 1986; Goggin and Lester, 1987).

A *Perkinsus* sp. similar to *P. marinus* was reported recently to be widespread in bivalve molluscs from the Italian coast (Daros and Canzonier, 1985). Mortalities of two species of clams, *Venerupis decussata* and *Venerupis aurea*, were associated with heavy infections and high prevalences of the parasite; other bivalves, including *Ostrea edulis* imported from Greece, were also found to be infected.

I. Summary

It is obvious from the preceding discussion that some of the Protozoa—notably members of the phyla Acetospora and Apicomplexa—are

important pathogens of molluscs, especially oysters. In fact, examining the total spectrum of molluscan diseases in this volume, it seems safe to state that the Protozoa (as presently defined) constitute the most significant pathogen group, in terms of the effects on populations.

The phylum Asctosporas, as presently delimited, contains a number of severe pathogens of oysters (and other bivalves); most of these disease-causing organisms have proved to be remarkably resistant to full understanding by humans. Despite extensive research by government and university scientists, some basic questions remain unanswered. With *Haplosporidium nelsoni*, for example, the method of transmission is unknown, as is the possible existence of an alternate host. With *Marteilia refringens* experimental transmission has not been achieved, and some disparity exists between the extent of infection and expected levels of mortality. On the other hand, much has been learned about life cycle stages and ecology, and empirical management techniques have been developed to enable survival of molluscan shellfish industries confronted with the continuing presence of the pathogens.

It is important to note, though, that there are still other pathogens of molluscs that must be described at present at "taxonomic orphans." Included in this category are presumed pathogens responsible for poorly defined diseases, and often placed in the genera *Labyrinthula* or *Labyrinthomyxa*. Thus, an organism seen in tissues of oysters (*Crassostrea virginica*) from Malpeque Bay, Canada, and considered a possible cause of earlier mortalities there has been described as a species of *Labyrinthula* (Li, 1976; Li and Clyburne, 1979); a *Labyrinthomyxa*-like organism, *Thanatostrea polymorpha*, was proposed earlier as a possible etiological agent of gill disease of *Crassostrea angulata* in Europe (Arvy and Franc, 1968; Franc and Arvy, 1969, 1970; Gutiérrez, 1977a); a mortality of mussels in the Gulf of St. Lawrence was attributed to the effects of a pathogen tentatively identified as *Labyrinthomyxa* sp. (Li and Clyburne, 1979); an organism described as "similar to *Dermocystidium marinum*" (*Perkinsus marinus*) was implicated in recurrent mass mortalities of bean clams, *Donax gouldi*, from the California coast (Coe, 1955); and a *Labyrinthomyxa*-like organism was isolated from heart tissues of *Crassostrea gigas* being cultivated in France (Cousserans *et al.*, 1974).

It seems quite likely that some of the above organisms may turn out to be asctosporans or apicomplexans (possibly *Perkinsus* spp.), some may be fungi, and some may not be pathogens at all. Thus, it would be prudent to leave a detailed description of these ill-defined organisms to some later treatise on molluscan diseases. This neglect does *not* mean that there may not be serious pathogens lurking in this assemblage—merely that data are insufficient to warrant more extensive treatment

here. It is always tempting, when mass mortalities occur, to identify a specific pathogen as quickly as possible, and the organisms described as "*Labyrinthula*-like" or "*Labyrinthomyxa*-like" are at times conspicuous, if baffling, parasites—which may or may not be the cause of the mortality being examined. It does appear, though, that "protistan" parasites (used in the broadest sense of the term) are the dominant pathogens of bivalve molluscs, and their importance should not be denigrated by the present inability of investigators to sort all of them out—especially presumed members of the protozoan phylum Labyrinthomorpha—and to assess the extent of damage they cause to host populations.

An encouraging recent trend in shellfish pathology has been the increased success in recognizing etiological agents in episodes in which infectious diseases have been involved. This has been especially true when ascetosporan or apicomplexan parasites are implicated, even though some of the Labyrinthomorpha-like organisms still defy scientific understanding. One recent example of the gradual emergence of knowledge about potential pathogens concerns a parasite of the oocytes of Pacific oysters, *Crassostrea gigas*. The organism was recognized (but identified only as an "amoeba-like protistan") by Imai *et al.* (1968), Matsusato *et al.* (1977), and Chun (1979). Subsequent studies with electron microscopy enabled determination of its taxonomic position as an ascetosporan of the class Paramyxia, and it has been described as *Marteilioides chungmuensis* (Comps *et al.*, 1986, 1987). It is probable that this is the same organism as that described (but not identified) two decades ago from *C. gigas* sampled on the California coast (Becker and Pauley, 1968).

Retrospective analyses of descriptions of some of the great oyster mortalities of the past have disclosed enticing similarities to some of the more recent ones, especially in the occurrence of certain disease signs and patterns of mortalities. In a few instances archived samples of preserved oysters have been retrieved from back shelves of museums, or faded histological slides have been found in about-to-be-discarded collections. Usually, though, yellowing documents reporting unsuccessful studies that were undertaken constitute the only remaining link with those past events—some of undeniably epic proportions—whose etiologies must forever remain matters of conjecture and speculation.

III. PROTOZOAN DISEASES OF CRUSTACEANS

Protozoa often cause chronic or acute disease in marine Crustacea. Among the most seriously pathogenic are microsporeans in shrimp and crabs, amoebae in crabs, and ciliates in shrimp and crabs (Couch, 1983).

Table 9. Principal Microsporean Diseases of Shrimp of North America

Host	Parasite	Disease Signs	Effects on Host	Geographic Distribution	References
<i>Penaeus setiferus</i> (white shrimp), <i>Penaeus duorarum</i> (pink shrimp)	<i>Agnasoma</i> (formerly <i>Thelohania</i>) <i>penaei</i>	Opaque white patches along dorsal midline; spores may fill hemocoel, destroy the gonads, and surround fibers of dorsal abdominal musculature	May cause parasitic castration in pink shrimp, thought responsible for extensive tissue destruction in white shrimp, may affect migratory behavior	Gulf of Mexico and south Atlantic coast of the United States	Viosca (1945), Sprague (1950a, 1970a), Hazard and Oldacre (1975), Lightner (1975), Kelly (1979)
<i>Penaeus aztecus</i> (brown shrimp), <i>P. duorarum</i> , <i>P. setiferus</i>	<i>Ameson</i> (formerly <i>Nosema</i>) <i>nelsoni</i>	Causes disease condition known as "cotton shrimp," resulting from surrounding of abdominal muscle fibers by masses of spores	Most common microsporean in wild penaeid shrimp of Gulf of Mexico, recognized as a threat to pond culture of shrimp, mortalities occur when infected shrimp are stressed	Gulf of Mexico and south Atlantic coast of the United States	Sprague (1950a, 1977, 1978), Overstreet (1973), Lightner (1975)
<i>Penaeus duorarum</i> ; also reported in <i>penaeus brasiliensis</i> , <i>P. setiferus</i> , and <i>P. aztecus</i>	<i>Thelohania duorara</i>	Also causes disease condition known as cotton shrimp; masses of spores in hemocoel, gut wall, and interstices of muscle bundles; destruction of muscle fibers rare	Mortalities may occur when infected shrimp are stressed	Dry Tortugas westward to Mississippi	Iversen and Manning (1959), Iversen and Van Meter (1964), Kelly (1979), Overstreet (1973)

<i>Penaeus aztecus</i> , <i>P. duorarum</i> , <i>P. setiferus</i>	<i>Pleistophora</i> sp.	Causes cotton shrimp condition similar to that caused by <i>A. nelsoni</i> ; abdominal muscles of host invaded and replaced by spores, occasionally in other tissues, dorsal abdomen with blue-black pigmentation	Mortalities may occur when infected shrimp are stressed	Gulf of Mexico and south Atlantic coast of the United States	Baxter <i>et al.</i> (1970), Constransitch (1970)
<i>Crangon stylirostris</i> , <i>C. alaskensis</i> , <i>C. franciscorum</i> , <i>C. nigricauda</i> , <i>C. nigronaculata</i> , <i>Pandalus jordani</i>	<i>Pleistophora crangoni</i>	Abdominal muscles soft, mushy, opaque white; opaque white streaks in muscle fibers of abdomen, appendages, and cephalothorax	In heavy infections parasitized muscle fibers are replaced by spores and normal fibers are distorted, entire muscle bundles may be destroyed, suppression of female gonadal development indicated	Pacific Northwest coast of the United States	Breed and Olson (1977), Sparks (1985)
<i>Pandalus jordani</i> (Pacific pink shrimp)	<i>Thelohania butleri</i>	Skeletal muscle opaque white, no light infections seen	Most common microsporidian parasite of <i>P. jordani</i> in area studied, density-dependent processes may affect infection levels	Pacific coast of the United States	Butler (1964), Johnston <i>et al.</i> (1978), Olson and Lan-nan (1984)
<i>Pandalus borealis</i> (northern shrimp)	<i>Microsporidium</i> sp. (generic group)	Skeletal muscle opaque white, no light infections seen	Muscle fibers invaded and necrotic, whole muscles not completely destroyed, prevalence in sampling area 0.1%, infected individuals incapable of reproduction	Labrador and western Greenland coast	Parsons and Khan (1986)

Because of their economic importance in many parts of the world, the decapod crustaceans have been examined extensively from the viewpoint of diseases, and a large body of literature has developed. Summaries of that literature have been published (Lightner, 1975; Couch, 1978, 1983; Overstreet, 1978; Sparks, 1985). Diseases of other crustacean groups have also received some scientific attention, but this chapter concentrates mostly on the decapods, beginning with shrimp and progressing to crabs and lobsters.

Serious pathogens of decapod crustaceans can be found in several protozoan phyla, as indicated in Table 6 at the beginning of this chapter. Certainly, members of the phylum Microspora must be considered a dominant group. Several genera of microsporeans parasitize shrimp and crabs, causing mortality or reducing market values (Couch, 1983; Sparks, 1985). A good example of this can be found in a listing of the major microsporean infections of shrimp from North America (Table 9).

A. Shrimp Diseases

Some of the most severe microsporean diseases of shrimp can be recognized grossly by a progressive white opacity of muscles and other tissues. The disease condition has been reported from shrimp of several genera (notably *Penaeus*, *Pandalus*, and *Crangon*). As an example, three different species of pathogens cause the abnormality known as "cotton shrimp" or "milky shrimp" in penaeids of the southwestern United States. One, *Ameson* (formerly *Nosema*) *nelsoni*, was described by Sprague (1950a) from the brown shrimp, *Penaeus aztecus*, in the Gulf of Mexico. Affected body muscles had an opaque white discoloration, and black pigment spots occurred externally (Fig. 19). The disease was common in bait shrimp as well as in those processed as food. Infected individuals did not survive well in bait tanks and were also discarded in processing plants, thus representing significant losses to the industry (Woodburn *et al.*, 1957; Hutton *et al.*, 1959; Overstreet, 1973). *Ameson nelsoni* infections appear to be chronic, gradually debilitating host animals. Body muscle fibers are first surrounded and then replaced by masses of spores (Lightner, 1975). Prevalences in pond-reared brown shrimp from Texas have been reported at 16%, and in white shrimp (*Penaeus setiferus*) in a net-enclosed Florida bay at 14%—suggesting a potential threat to shrimp reared in extensive culture (Lightner, 1975).

Another microsporean causing a similar cotton shrimp condition is *Thelohania duorara*, described by Iversen and Manning (1959) from the musculature of pink shrimp, *Penaeus duorarum*, from Florida waters of the Gulf of Mexico. Infected individuals were relatively rare, however, in



Figure 19. "Cotton shrimp" (bottom) infected with *Ameson nelsoni*, compared with normal shrimp (top). (Courtesy of Dr. D. V. Lightner.)

landed catches. Brazilian brown shrimp, *Penaeus brasiliensis*, harbor the same parasite (Iversen and Van Meter, 1964; Overstreet, 1973). Spores develop between muscle fibers, but may eventually replace them. Infections may also occur in heart, nerves, and gonads (Lightner, 1975).

Pleistophora sp. is still another microsporean which causes the cotton shrimp condition, especially in brown shrimp, *P. aztecus*, of the Gulf of Mexico, but in other species as well. Gross signs (i.e., muscle opacity) are not distinguishable from those caused by *A. nelsoni* or *T. duorara* (Baxter *et al.*, 1970; Overstreet, 1973). The pathogen typically invades and replaces body muscles, but may occur also in the gills, hepatopancreas, stomach, and heart. *Pleistophora* sp. was less common than *A. nelsoni* in shrimp sampled on the Texas coast; it occurred in only 6% of infected shrimp in one study and was not seen in cultured shrimp (Lightner, 1975).

A fourth microsporean, *Agmasoma* (formerly *Thelohania*) *penaei*, from Gulf of Mexico white shrimp, *P. setiferus*, affects the gonads and other tissues rather than the body muscles (Sprague, 1950a) (Fig. 20). Earlier, Viosca (1945) reported that "about 90% of white shrimp in Louisiana waters were infected in 1919 by a protozoan disease [not further identified] which destroyed the reproductive organs." If the disease were caused by *A. penaei*, the microsporean may play a role in fluctuations of



Figure 20. *Agmasoma penaei* infection of the gonad in *Penaeus setiferus*. (Courtesy of Dr. S. K. Johnson.)

the host species when present at epizootic levels. The spores of this parasite are usually located in the median dorsal region of the host, invading gonads, foregut, and blood vessels (Overstreet, 1973). Production of eggs and sperm is suppressed or eliminated in diseased individuals.

The opacity of abdominal muscles characteristic of several of the cotton shrimp diseases, caused by microsporeans in penaeids, has counterparts in other genera in other bodies of water. Pandalid shrimps *Pandalus borealis* from the northwest Atlantic and *Pandalus jordani* from the northeast Pacific are infected by *Microsporidium* sp. (generic group) and *Thelohania butleri* respectively—with muscle opacity the predominant gross sign of disease (Olson and Lannan, 1984; Parsons and Khan, 1986). Other species of *Thelohania* have been reported from the body muscles of European shrimp (Henneguy and Thélohan, 1892; Gurley, 1893).

While microsporean infections can cause obvious and severe pathology in shrimp, effects on host population abundance are mostly undetermined. Destruction of gonads may lead to a reduction in reproductive potential—as seen in *Agmasoma penaei* parasitization—and muscle opacity must lead to greater vulnerability to predation, but population effects are difficult to detect in feral populations. Infected indi-

viduals do not survive well in captivity and die quickly if handled, suggesting a poor general response to environmental stressors. No definitive evidence of mass mortalities of shrimp attributable to microsporean parasitization has been reported, but there is some suggestion that migratory behavior may be affected, with infected individuals failing to participate in normal offshore movements (Overstreet, 1973). There is also a speculation that microsporean infections may be lethal to juvenile shrimp (Overstreet, 1973).

Several gregarines (phylum Apicomplexa) occur in shrimp. Sprague (1954) tentatively identified as *Nematopsis penaeus* a gregarine from the digestive tract of brown shrimp from Louisiana, and mentioned the possibility of extensive damage to the intestinal epithelium of heavily infected individuals. The same parasite was observed by Kruse (1959a-c) and by Hutton *et al.* (1959) in several species of shrimp from Florida. Another species, *Nematopsis duorari*, was identified and described by Kruse (1966) from the digestive tract of Florida pink shrimp. Kruse demonstrated by infection experiments that shrimp become infected by eating mucus strings from bivalve molluscs in which the spore stages occur.

Kruse (1959a,b) erected a new genus, *Cephalolobus*, for a gregarine, *Cephalolobus penaeus*, from the chitinous lining of the stomach of pink and brown shrimp of the northern Gulf coast of Florida. Another member of the genus, *Cephalolobus petiti*, infects commercial shrimp, *Solenocera membranacea*, from the French Mediterranean coast (Théodorides, 1962).

Ciliates also occur as parasites and epibionts of shrimps. The peritrich, *Lagenophrys lunatus*, was described by Imamura (1940) from *Leander squilla* in Japan. The apostome, *Terebrospira lenticularis*, an ectoparasite which perforates the exoskeleton of *Palaemon varians*, was identified and studied by Debaisieux (1960). A complex life cycle, intimately related to molting of the host, was capable of producing heavy parasitization in restricted environments. Stalked ciliates, especially those of the genus *Zoothamnium*, can be problems in high-density shrimp culture. Extensive colonization of gills may interfere with gas exchange and result in mortalities (Overstreet, 1973; Lightner, 1975; Couch, 1978, 1983).

B. Crab Diseases

Crabs, particularly the brachyurans, are of economic importance in many countries. Wild populations are subject to a number of protozoan diseases, some of which occur in epizootic proportions, with resulting mass mortalities. Usually, though, protozoan diseases are enzootic, kill-

ing the occasional individual. Often, too, diseases become apparent only when crabs are being held in tanks or live cars for market.

Microsporeans are dominant among protozoan pathogens of crabs, just as in shrimp. In fact, some of the same genera which contain shrimp parasites also include species known as crab parasites: *Ameson* (formerly *Nosema*), *Thelohania*, and *Pleistophora*. Additionally, a few members of the phyla Sarcomastigophora (e.g., *Paramoeba* and *Hematodinium*) and Ciliophora (*Paranophrys*) have become recognized as crab pathogens.

Ameson michaelis is a microsporean which infects muscles of the blue crab, *Callinectes sapidus*, on the Gulf and Atlantic coasts of the United States (Sprague, 1970a, 1977). The disease causes lysis and necrosis of muscle tissue, transmission is direct, and infections may be lethal (Sprague, 1965a; Vernick and Sprague, 1970; Weidner, 1970; Overstreet, 1978). Gross signs of heavy infections include lethargic movements, opaque white musculature visible through the thin exoskeleton at joints of appendages, and rapid mortality when diseased animals are stressed (Overstreet and Whatley, 1975; Overstreet, 1977). Another species of *Ameson*, *A. pulvis*, has been reported in the European shore crabs *Carcinus maenas* and *Carcinus mediterraneus* from France (Pérez, 1905a,b; Vivares and Sprague, 1979). Like *A. michaelis*, it infects the body muscles, but other effects have not been described.

Members of a second microsporean genus, *Thelohania*, also cause muscle destruction in crabs. *Thelohania maenadis* infects *C. maenas* and *C. mediterraneus* on the French coast (Pérez, 1904, 1907; Sprague, 1978), causing a typical white opacity and extensive destruction of muscle tissue (Vivares and Cuq, 1981). Another species, *Thelohania petrolisthis*, causes muscle degeneration and mortality in the crab *Petrolisthes armatus* from the Gulf of Mexico (Sprague, 1970a). Still other species of *Thelohania* infect king crabs *Paralithodes camtschatica* and *Paralithodes platypus* from Alaskan waters (Sparks, 1985).

A third microsporean genus, *Pleistophora*, is represented only weakly among the crab pathogens—with a single species, *Pleistophora cargoii*, which occasionally parasitizes the muscles of the blue crab, *C. sapidus*, from Chesapeake Bay (Sprague, 1966, 1970a).

Protozoan phyla other than the Microspora contain a few serious pathogens of crabs. The phylum Sarcomastigophora, for example, contains an amoebic parasite, *Paramoeba pernicioso*, and a parasitic dinoflagellate, *Hematodinium perezi*—both of which cause lethal infections in blue crabs, *C. sapidus*, from the Atlantic coast of the United States.

The disease caused by *P. pernicioso* is often called gray crab disease, because some infected individuals develop a definite grayish cast to the normally ivory-colored ventral exoskeleton (Fig. 21). Diseased blue crabs



Figure 21. Paramoebiasis in an infected (top) and normal (bottom) blue crab. Note the difference in ventral coloration.

become lethargic in late spring, they die quickly when handled, and mass mortalities occur in May and June of epizootic years (Sprague and Beckett, 1966, 1968; Sawyer, 1969; Sprague *et al.*, 1969; Newman and Ward, 1973; Johnson, 1977; Couch, 1983). The parasite occurs in the hemolymph and the connective tissues of the host; in heavy infections hemocytes are replaced by amoebae throughout the circulatory system; and massive invasion, lysis, and replacement of connective tissue occurs. Death ensues, probably as a consequence of the drastic decline in hemolymph glucose and serum proteins, combined with destruction of vital tissues and occlusion of the gill sinuses (Pauley *et al.*, 1975). Prevalences of up to 20% were found in some samples of crabs taken from the Middle Atlantic states in 1969 (Couch, 1983). Peak prevalences occurred in May–June and (in dredged hibernating crabs) in October–February. The disease in epizootic form could thus be a cause of annual winter and

late spring mortalities in blue crabs (Couch, 1983) and the cause of chronic low-level mortalities in late spring of nonepizootic years. Marked annual and geographic variations in prevalences occur, and mortalities have been most common in small coastal bays from Maryland to Georgia. The pathogen appears to be opportunistic, invading through injuries to the host's exoskeleton—especially that of newly molted individuals. It also occurs in rock crabs, *Cancer irroratus*, and lobsters, *Homarus americanus* (Sawyer and MacLean, 1978).

Another member of the protozoan phylum Sarcomastigophora—*Hematodinium perezii*—has been described as a severe pathogen of blue crabs (Newman and Johnson, 1976). This parasitic dinoflagellate causes mortality, but provides no external signs of disease except for lethargy and weakness of infected individuals prior to death. It occurs primarily in the hemolymph (which develops a cloudy, opalescent appearance) but may invade and lyse body muscles and hepatopancreatic tubules in advanced infections (Couch, 1983). The organism seems salinity limited: Infections have not been found in waters below 11 ‰. *Hematodinium perezii* has also been reported to infect *Carcinus maenas* and *Portunus depurator* from the French coast (Chatton and Poisson, 1930), as well as *Ovalipes ocellatus*, *Cancer irroratus*, and *C. borealis* from the Middle Atlantic coast of the United States (MacLean and Ruddell, 1978). Additionally, systemic dinoflagellate infections were reported recently from tanner crabs, *Chionoecetes bairdi*, from Alaskan waters (Meyers *et al.*, 1987). Known as bitter crab disease, the infection is chronic but lethal, and also imparts a bitter flavor to processed meats. Prevalence in one sampling area was 95% in 1985–1986, and two thirds of the major crab producing areas in southeastern Alaska were affected.

Lethal infections of crabs by ciliates (members of the protozoan phylum Ciliophora) have been reported sporadically from Europe and North America. The genus *Paranophrys* contains opportunistic pathogens which invade through damaged exoskeleton and proliferate in the hemolymph and the body tissues of the host, eventually killing it. The earliest description was of *Paranophrys* (formerly *Anophrys*) *maggii*, parasitizing the European shore crab *Carcinus maenas* (Cattaneo, 1888). The organism reappeared in wild crabs on the French coast (Poisson, 1929, 1930) and as a cause of mortality in *Cancer pagurus* populations in commercial holding tanks in France (Bang *et al.*, 1972). The most recent reports describe *Paranophrys* infections of wild and captive Dungeness crabs, *Cancer magister*, on the Oregon coast of the United States (Armstrong *et al.*, 1981; Sparks *et al.*, 1982; Hibbits and Sparks, 1983).

The disease of Dungeness crabs caused by *Paranophrys*—lethal in its outcome—is characterized by invasion of the hemolymph and prolifera-

tion of the ciliates, coincident with the disappearance of hemocytes. Extensive tissue disruption, particularly of the heart, occurs in advanced infections. Other organs—eyestalks, stomach, gills, and body muscles—may also be infected and damaged. Death appears to be a consequence of this massive tissue destruction.

Captive crabs seem particularly vulnerable to ciliate infection, especially if they have been injured during capture or transfer, but the disease occurs in feral crabs as well (Poisson, 1930; Sparks *et al.*, 1982). That the same or closely related ciliates infect other crustaceans has been demonstrated in several recent reports. American lobsters, *Homarus americanus*, from holding tanks in Canada, died from severe *Paranophrys* infections (Aiken *et al.*, 1973), and later studies disclosed infections in wild stocks. Experimental infections were achieved by injecting hemolymph from diseased individuals, and death occurred within 6–8 weeks postinjection, in water temperatures from 0 to 5°C (Aiken and Waddy, 1986). Additionally, marine isopods, *Gnorimosphaeroma oregonensis*, from Alaska had massive, systemic *Paranophrys* infections at what were described as epizootic levels (Hibbits and Sparks, 1983).

Members of the apostome ciliate genus *Synophrya* may damage the gills of crabs. Several studies, beginning with those by Chatton and Lwof (1926, 1927, 1935) and including those by Johnson and Bradbury (1976), have demonstrated the pathogenicity of *Synophrya* to crabs, with high prevalences (up to 75%) in some areas sampled. Gills may be blackened and eroded, with pronounced host response and extensive necrosis.

Ciliates may be, on occasion, significant epibionts of crabs. As an example, molting blue crabs from Chesapeake Bay suffered serious mortalities in the summers of 1965 and 1966. Their gills had a massive infestation of peritrichous ciliates (Fig. 22) of the genera *Lagenophrys* and *Epistylis* (Couch, 1966, 1967a). Mortalities were most severe among crabs in holding tanks just before or after molting, but wild crabs were also heavily infested, and fishermen reported mortalities. Infestations of gills frequently seemed heavy enough to interfere with respiration. Although these gill ciliates are not technically parasitic, in that they do not derive nourishment directly from the host, they are of obvious detriment to the crab.

Gregarines (phylum Apicomplexa) are common parasites of many Crustacea, and an extensive literature about them has accumulated. Many members of the group have been reported from crabs. For example, species of the genus *Cephaloidophora* occur in spider and fiddler crabs of the U.S. East Coast (Watson, 1915, 1916a,b; Watson-Kamm, 1922); in the striped shore crab, *Pachygrapsus crassipes*, of the Pacific coast; and in the Mediterranean flat crab, *Pachygrapsus marmoratus* (Ball, 1938; Thé-

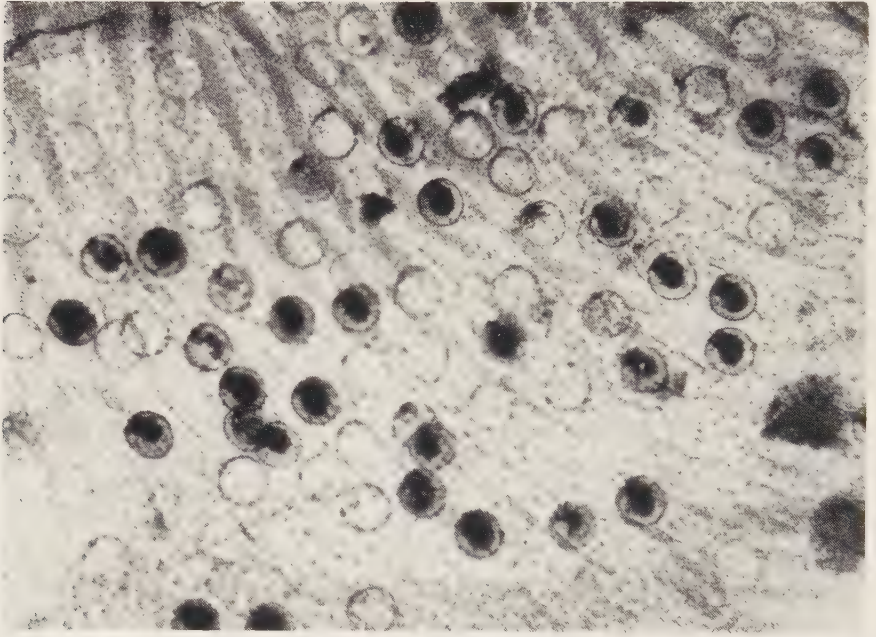


Figure 22. Infestation of the gills of a blue crab by the ciliate *Lagenophrys*. (Courtesy of Dr. J. A. Couch.)

odorides, 1961, 1962). As mentioned in the discussion of oyster diseases, several representatives of the genus *Nematopsis* occur in Atlantic species of mud crabs (Prytherch, 1940; Ball, 1951; Sprague and Orr, 1955).

Although the gregarines are not usually considered serious pathogens of Crustacea, masses of the parasites may occlude the lumen of the intestinal caeca and may damage the epithelium. Ball (1938) found that the lumen of the intestinal caeca of the crab *P. marmoratus* could be practically occluded by *Carcinoecetes conformis* and that a thinning, and even sloughing, of the epithelium occurred. A similar sloughing was noted in *Panopeus occidentalis* and *Panopeus herbstii* due to activities of *Nematopsis panopei* (Ball, 1951).

Earlier in this chapter, the point was made that the protozoan phylum Ascomycota contained a number of severe pathogens of molluscs. It is interesting, therefore, that this phylum is poorly represented among the pathogens of crustaceans. *Minchinia* (now *Haplosporidium*) *louisiana* was described by Sprague (1963a) in the mud crab, *P. herbstii*, taken from the Louisiana coast. Life cycle stages, including definitive spores, were identified from heavy infections of the entire gut wall. A second as-

cetosporan (haplosporidan), not further identified, was reported from blue crabs of the Middle Atlantic coast of the United States (Newman *et al.*, 1976). The disease was rare, but infections were apparently lethal. Masses of plasmodia of the pathogen were distributed throughout the vascular spaces, and the hemolymph was opaque white, similar to that seen in *Paramoeba* disease, described earlier.

C. Lobster Diseases

Except for the effects of the ciliate *Paranophrys* described in the previous section, it is difficult to find examples of pathogenic roles for Protozoa in lobster populations. No dominant organism, such as the bacterium *Aerococcus viridans* var. *homari* (described in Chapter 3), emerges among protozoan parasites of lobsters, and we are left with only "minor-league" candidates.

- Dannevig (1928, 1939), in reports on Norwegian lobster hatcheries, described infection and destruction of eggs on the females by the suctorian (phylum Ciliophora) *Ephelota gemmipara*. The protozoan was found on newly caught individuals and increased tremendously on lobsters in hatching boxes. Dannevig attributed substantial (90%) decreases in the production of larvae to the effects of the parasite. The organism was abundant only in certain years.
- The gregarine protozoan, *Porospora gigantea*, has been reported as parasitic in the digestive tract of the European lobster (Hatt, 1928, 1931), and was found in all of 202 American lobsters from the Magdalen Islands, Gulf of St. Lawrence, Canada, by Montreuil (1954). A later parasitological survey of lobsters from the Gulf of St. Lawrence disclosed a similarly high (84%) prevalence of *P. gigantea*, encysted in numbers up to 500 in the walls of the digestive tract (Boghen, 1978). Another species, *Porospora nephropis*, has been described in the Norway lobster, *Nephrops norvegicus*, by Léger and Duboscq (1915) and by Tuzet and Ormieres (1961).
- A fatal and apparently infectious disease of *Panulirus argus* from Florida waters was observed by Sims (1966). Affected individuals became disoriented and their abdomens were "milky", a condition reminiscent of microsporean infections of shrimp (Sprague, 1950a) and freshwater crayfish (Sprague, 1950b; Sogandares-Bernal, 1962), as was pointed out by Sims.

It seems highly unlikely that this sparse representation of the Protozoa among pathogens of lobsters is a reflection of the true state of

affairs, particularly in view of the importance of a number of protozoan pathogens in other decapods, such as shrimps and crabs. New research will undoubtedly augment available information.

D. Summary

Examining the foregoing brief description of protozoan parasites of Crustacea, it is possible to discern four categories:

1. Some, such as the Microspora, are obligately parasitic and often lethal.
2. Some severe pathogens are probably opportunistic invaders, normally free living, which penetrate injured hosts and multiply in hemolymph and tissues; this category includes the ciliate *Paranophrys* (formerly *Anophrys*) spp. and the amoeba, *Paramoeba perniciosa*.
3. Some internal parasites, such as the gregarines, seem well adapted to their crustacean hosts and cause obvious pathology only if present in overwhelming numbers.
4. Some, such as the ciliates *Lagenophrys callinectes* and *Zoothamnium* sp., normally commensals or epibionts, may affect host survival if present in large numbers.

Lethality of some protozoan infections has been demonstrated in feral and captive populations; additional stresses imposed by capture and holding frequently disclose preexisting disease conditions in wild populations.

IV. CONCLUSIONS

This brief examination of principal protozoan diseases of shellfish has resulted in the reinforcement of several general findings:

- Among the protozoan pathogens of shellfish, dominant groups are the Ascetospora (for molluscs) and the Microspora (for crustaceans). Ascetosporan genera of significance in molluscs are *Haplosporidium*, *Marteilia*, and *Bonamia*. Microsporean genera of importance in crustaceans are *Ameson* (formerly *Nosema*), *Thelohanina*, and *Pleistophora*. Severe pathology, tissue destruction, and mortality can be consequences of infection.
- Epizootics due to virulent protozoan pathogens have been a continuing phenomenon among oyster populations in several parts of the world during the past four decades, in the United States,

Europe, and Australia. Whereas some of the pathogens (notably members of the phylum Labyrinthomorpha) remain enigmatic, others (such as members of the phyla Ascomycota and Apicomplexa) have been examined scientifically in great detail, with generally satisfying results.

- From the studies conducted, epizootics and resultant mass mortalities have clearly been shown to reduce population abundance of oyster stocks and have forced development of alternate methods of culture.
- Several recent epizootics in oysters have been characterized by continuing high prevalences of the protozoan pathogens and continuing high mortality levels after the initial epizootic peak, but also by indications of increasing resistance among surviving populations.
- Among Crustacea, widespread and long-term disease outbreaks comparable to *Krebspest* of European freshwater crayfish (caused by the fungus *Aphanomyces astaci*) have not occurred among feral marine species, but more localized epizootics, such as gray crab disease (caused by the amoeba *Paramoeba perniciosus*), have been observed and reported.

It can reasonably be concluded from examining available literature on protozoan diseases of shellfish that this group of pathogens is probably the most significant of any comparable group in its effects on molluscan and crustacean hosts.

REFERENCES

- Aiken, D. E., and Waddy, S. L. (1986). Environmental influence on recruitment of the American lobster, *Homarus americanus*: A perspective. *Can. J. Fish. Aquat. Sci.* **43**, 2258–2270.
- Aiken, D. E., Sochasky, J. B., and Wells, P. G. (1973). Ciliate infestation of the blood of the lobster, *Homarus americanus*. *Int. Counc. Explor. Sea, Doc. C.M.1973/K:46*, 2 pp.
- Alder, J., Braley, R., and Shinwari, W. (1986). An account of serious mortality in the population of giant clams on reefs surrounding Lizard Island. *Rep. Queensl. Natl. Parks Wildl. Serv., Cairns* (cited by Goggin and Lester, 1987).
- Alderman, D. J. (1979). Epizootiology of *Marteilia refringens* in Europe. *Mar. Fish. Rev.* **41**(1–2), 67–69.
- Andrews, J. D. (1955). Notes on fungus parasites of bivalve mollusks in Chesapeake Bay. *Proc. Natl. Shellfish. Assoc.* **45**, 157–163.
- Andrews, J. D. (1964). Oyster mortality studies in Virginia. IV. MSX in James River public seed beds. *Proc. Natl. Shellfish. Assoc.* **53**, 65–84.
- Andrews, J. D. (1965). Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. *Chesapeake Sci.* **6**, 60–67.

- Andrews, J. D. (1966). Oyster mortality studies in Virginia. V. Epizootiology of MSX, protistan pathogen of oysters. *Ecology* **47**, 19–31.
- Andrews, J. D. (1967a). Oyster mortality studies in Virginia. VI. History and distribution of *Minchinia nelsoni*, a pathogen of oysters, in Virginia. *Chesapeake Sci.* **8**, 1–13.
- Andrews, J. D. (1967b). Interaction of two diseases of oysters in natural waters. *Proc. Natl. Shellfish Assoc.* **67**, 38–49.
- Andrews, J. D. (1968). Oyster mortality studies in Virginia. VII. Review of epizootiology and origin of *Minchinia nelsoni*. *Proc. Natl. Shellfish. Assoc.* **58**, 23–36.
- Andrews, J. D. (1976a). Epizootiology of oyster pathogens *Minchinia nelsoni* and *M. costalis*. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st pp. 169–171.
- Andrews, J. D. (1976b). Epizootiology of *Dermocystidium marinum* (= *Labyrinthomyxa marina*) in oysters. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st pp. 172–174.
- Andrews, J. D. (1977a). Delaware Bay disease of oysters. In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann, ed.), pp. 204–210. Elsevier, Amsterdam.
- Andrews, J. D. (1977b). Seaside disease of oysters, In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann, ed.), pp. 211–214. Elsevier, Amsterdam.
- Andrews, J. D. (1979). Oyster diseases in Chesapeake Bay. *Mar. Fish. Rev.* **41**, 45–53.
- Andrews, J. D. (1984). Epizootiology of haplosporidan diseases affecting oysters, *Comp. Pathobiol.* **7**, 243–269.
- Andrews, J. D. and Castagna, M. (1978). Epizootiology of *Minchinia costalis* in susceptible oysters in seaside bays of Virginia's eastern shore 1959–1976. *J. Invertebr. Pathol.* **32**, 124–138.
- Andrews, J. D., and Frierman, M. (1974). Epizootiology of *Minchinia nelsoni* in susceptible wild oysters in Virginia, 1959 to 1971. *J. Invertebr. Pathol.* **24**, 127–140.
- Andrews, J. D., and Hewatt, W. G. (1954). Incidence of *Dermocystidium marinum*, Mackin, Collier, and Owen, a fungus disease of oysters in Virginia. *Conv. Addresses Natl. Shellfish. Assoc.* 1953 p. 79 (abstract).
- Andrews, J. D., and Hewatt, W. G. (1957). Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. *Ecol. Monogr.* **27**, 1–26.
- Andrews, J. D., and Wood, J. L. (1967). Oyster mortality studies in Virginia. VI. History and distribution of *Minchinia nelsoni*, a pathogen of oysters, in Virginia. *Chesapeake Sci.* **8**, 1–13.
- Andrews, J. D., Wood, J. L., and Hoese, H. D. (1962). Oyster mortality studies in Virginia. III. Epizootiology of a disease caused by *Haplosporidium costale* Wood and Andrews. *J. Insect Pathol.* **4**, 327–343.
- Armstrong, D. A., and Armstrong, J. L. (1974). A haplosporidan infection in gaper clams, *Tresus capax* (Gauld), from Yaquina Bay, Oregon. *Proc. Natl. Shellfish. Assoc.* **64**, 68–72.
- Armstrong, D., Bureson, E. and Sparks, A. (1981). A ciliate infection (*Paranophrys* sp.) in laboratory-held Dungeness crabs, *Cancer magister*. *J. Invertebr. Pathol.* **32**, 201–209.
- Arvy, L., and Franc, A. (1968). Sur un protiste nouveau, agent de destruction des branchies et des palpes de l'huître portugaise. *C. R. Hebd. Seances Acad. Sci., Ser. D* **167**, 103–105.
- Bachere, E., and Grizel, H. (1982). Mise en évidence d'*Haplosporidium* sp. (Haplosporida, Haplosporidiidae) parasite de l'huître plate *Ostrea edulis* L. *Rev. Trav. Inst. Pêches Marit.* **46**(3), 226–232.
- Ball, G. H. (1938). The life history of *Carcinoecetes hesperus* n. gen., n. sp., a gregarine parasite of the striped shore crab, *Pachygrapsus crassipes*, with observations on related forms. *Arch. Protistenkd.* **90**, 299–319.

- Ball, G. H. (1951). Gregarines from Bermuda marine crustaceans. *Univ. Calif., Berkeley, Publ. Zool.* **47**, 351–368.
- Balouet, G. (1979). *Marteilia refringens*—considerations of the life cycle and development of Aber disease in *Ostrea edulis*. *Mar. Fish. Rev.* **41**(1–2), 64–66.
- Balouet, G., Cahour, A., and Chastel, C. (1979). Epidémiologie de la maladie de la glande digestive de l'huître plate: Hypothèses sur le cycle de *Marteilia refringens*. *Haliotis* **8**, 323–326.
- Balouet, G., Poder, M., and Cahour, A. (1983). Haemocytic parasitosis: Morphology and pathology of lesions in the French flat oyster, *Ostrea edulis* L. *Aquaculture* **34**, 1–14.
- Bang, F. B., Audouin, J., and Leglise, M. (1972). Ciliate infection of the blood of the edible crab, *Cancer pagurus*, in holding tanks in Brittany, France. *J. Invertebr. Pathol.* **20**, 226–227.
- Barrow, J. H., Jr., and Taylor, B. C. (1966). Fluorescent-antibody studies of haplosporidan parasites of oysters in Chesapeake and Delaware Bays. *Science* **153**, 1531–1533.
- Baxter, K. N., Rigdon, R. H., and Hanna, C. (1970). *Pleistophora* sp. (Microsporidia: Nosematidae): A new parasite of shrimp. *J. Invertebr. Pathol.* **16**, 289–291.
- Becker, C. D., and Pauley, G. B. (1968). An ovarian parasite (*protista incertae sedis*) from the Pacific oyster, *Crassostrea gigas*. *J. Invertebr. Pathol.* **12**, 425–437.
- Boghen, A. D. (1978). A parasitological survey of the American lobster *Homarus americanus* from the Northumberland Strait, southern Gulf of St. Lawrence. *Can. J. Zool.* **56**, 2460–2462.
- Breed, G. M., and Olson, R. E. (1977). Biology of the microsporidan parasite *Pleistophora crangoni* n. sp. in three species of crangonid sand shrimps. *J. Invertebr. Pathol.* **30**, 387–405.
- Brehélin, M., Bonami, J.-R., Cousserans, F. C. and Vivares, C. P. (1982). Pathologie animale—Existence de formes plasmodiales vraies chez *Bonamia ostreae* parasite de l'huître plate *Ostrea edulis*. *C. R. Hebd. Séances Acad. Sci., Ser. III* **295**, 45–48.
- Bucke, D., and Feist, S. (1985). Bonamiasis in the flat oyster, *Ostrea edulis*, with comments on the histological techniques, In "Fish and Shellfish Pathology" (A. E. Ellis, ed.), pp. 387–392. Academic Press, London.
- Bucke, D., Hepper, B., Key, D., and Bannister, C. A. (1984). A report on *Bonamia ostreae* in *Ostrea edulis* in the U.K. *Int. Counc. Explor. Mer, Doc. C.M.* **1984/K:9**, 7 pp.
- Butler, T. H. (1964). Growth, reproduction and distribution of pandalid shrimp in British Columbia. *J. Fish. Res. Board Can.* **21**, 1403–1452.
- Cahour, A. (1979). *Marteilia refringens* and *Crassostrea gigas*. *Mar. Fish. Rev.* **41**(1–2), 19–20.
- Cahour, A., Poder, M., and Balouet, G. (1980). Présence de *Minchinia armoricana* (Haplosporea, Haplosporida) chez *Ostrea edulis* d'origine française. *C. R. Soc. Biol.* **174**(3), 359–368.
- Cattaneo, G. (1888). Su di un infusorio ciliato, parassito del sangue del *Carcinus maenas*. *Zool. Anz.* **11**, 456–459.
- Certes, A. (1882). Note sur les parasites et les commensaux de l'huître. *Bull. Soc. Zool. Fr.* **7**, 347–353.
- Chatton, E., and Lwoff, A. (1926). Les *Synophrya* infusoires parasites internes des crabes. Leur évolution à la mue. Leur place parmi les Foettingeriidae. *C. R. Acad. Sci.* **183**, 1131–1143.
- Chatton, E., and Lwoff, A. (1927). Le cycle évolutif de la *Synophrya hypertrophica* cilié Foettingeriidae. *C. R. Acad. Sci.* **185**, 877–879.
- Chatton, E., and Lwoff, A. (1935). Les ciliés apostomes. I. Aperçu historique et général; études monographiques des genres et des espèces. *Arch. Zool. Exp. Gén.* **77**(Fasc. 1), 1–453.
- Chatton, E., and Poisson, R. (1930). Sur l'existence dans le sang des crabes, de peridiniens parasites: *Hematodinium perezii* n. g., n. sp. (Syndinidae). *C. R. Soc. Biol.* **105**, 553–557.

- Chun, S. K. (1979). Amoeba infection in the oyster (*Crassostrea gigas*). *Bull. Korean Fish. Soc.* **12**(4), 281–285.
- Coe, W. R. (1955). Ecology of the bean clam *Donax gouldi* on the coast of southern California. *Ecology* **36**, 512–514.
- Comps, M. (1970). Observations sur les causes d'une mortalité anormale des huîtres plates dans le bassin de Marennes. *Rev. Trav. Inst. Pêches Marit.* **34**, 317–326.
- Comps, M. (1982). Recherches sur un protiste, parasite nouveau de l'huître plate des côtes françaises. *Malacologia* **22**, 1–2.
- Comps, M., Grizel, H., Tige, G., and Duthoit, J. -L. (1975). Parasites nouveaux de la glande digestive des mollusques marins *Mytilus edulis* L. et *Cardium edule* L. C. R. *Hebd. Séances Acad. Sci., Ser. D* **281**, 179–181.
- Comps, M., Tige, G., and Grizel, H. (1980). Pathologie animale—étude ultrastructurale d'un protiste parasite de l'huître *Ostrea edulis* L. C. R. *Hebd. Séances Sci., Ser. D* **290**, 383–385.
- Comps, M., Pichot, Y., and Papagianni, P. (1982). Recherche sur *Marteilia maurini* n. sp. parasite de la moule *Mytilus galloprovincialis* Lmk. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **45**, 211–214.
- Comps, M., Park, M. S., and Desportes, I. (1986). Étude ultrastructurale de *Marteilioides chungmuensis* n. g., n. sp., parasite des ovocytes de l'huître *Crassostrea gigas* Th. *Protistologica* **22**(3), 279–285.
- Comps, M., Park, M. S., and Desportes, I. (1987). Fine structure of *Marteilioides chungmuensis* n. g., n. sp., a parasite of the oocytes of the oyster *Crassostrea gigas*. *Aquaculture* **67**, 264–265.
- Constransitch, M. J. (1970). "Description, Pathology, and Incidence of *Plistophora penaei* n. sp. (Microsporida, Nosematidae) a Parasite of Commercial Shrimp," thesis. Northwestern University, Evanston, Illinois.
- Couch, J. A. (1966). Two peritrichous ciliates from the gills of the blue crab. *Chesapeake Sci.* **7**, 171–176.
- Couch, J. A. (1967a). A new species of *Lagenophrys* (Ciliata: Peritrichida: Lagenophryidae) from a marine crab, *Callinectes sapidus*. *Trans. Am. Micros. Soc.* **86**, 204–211.
- Couch, J. A. (1967b). Concurrent haplosporidian infections of the oyster, *Crassostrea virginica* (Gmelin). *J. Parasitol.* **53**, 248–253.
- Couch, J. A. (1978). Diseases, parasites and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North America. *Fish. Bull.* **76**, 1–44.
- Couch, J. A. (1983). Diseases caused by Protozoa, In "The Biology of Crustacea: Vol. 6 Pathobiology" (A. J. Provenzano, ed.), pp. 79–111. Academic Press, New York.
- Couch, J. A., and Rosenfield, A. (1968). Epizootiology of *Minchinia costalis* and *Minchinia nelsoni* in oysters introduced into Chincoteague Bay, Virginia. *Proc. Natl. Shellfish. Assoc.* **58**, 51–59.
- Couch, J. A., Farley, C. A., and Rosenfield, A. (1966). Sporulation of *Minchinia nelsoni* (Haplosporida, Haplosporidiidae) in *Crassostrea virginica* (Gmelin). *Science* **153**, 1529–1531.
- Cousserans, F., Bonami, J.-R., Comps, M., and Grizel, H. (1974). Développement de protistes parasites en culture de l'huître japonaise *Crassostrea gigas* Th. C. R. *Hebd. Séances Acad. Sci., Ser. D* **179**, 2119–2121.
- Dannevig, A. (1928). Beretning om Flødevigens utklekningsanstalt 1926–1927. *Aarsberet. Norg. Fisk.* pp. 150–156.
- Dannevig, A. (1939). Beretning om Flødevigens utklekningsanstalt 1936–1937. *Aarsberet. Norg. Fisk.* pp. 70–75.
- Daros, L., and Canzonier, W. J. (1985). *Perkinsus*, a protistan threat to bivalve culture in the Mediterranean basin. *Bull. Eur. Assoc. Fish Pathol.* **5**(2), 23–25.

- Debaissieux, P. (1960). Ciliates apostomes parasites de *Palaemon*. *Cellule* **60**, 333–352.
- DeVincentiis, M. and Renzoni, A. (1963). Sulla presenza di uno sporozoo in ovociti di *Mytilus galloprovincialis* Lam. *Arch. Zool. Ital.* **47**, 21–26.
- Dinamani, P., Hine, P. M., and Jones, J. B. (1987). Occurrence and characteristics of the haemocyte parasite *Bonamia* sp. in the New Zealand dredge oyster *Tiostrea lutaria*. *Dis. Aquat. Org.* **3**, 37–44.
- Elston, R. A., Farley, C. A., and Kent, M. L. (1986). Occurrence and significance of bonamiasis in European flat oysters *Ostrea edulis* in North America. *Dis. Aquat. Org.* **2**, 49–54.
- Engle, J. B., and Rosenfield, A. (1963). Progress in oyster mortality studies. *Proc. Annu. Sess. Gulf Caribb. Fish. Inst.* **15**, 116–124.
- Farley, C. A. (1965). Pathologic responses of the oyster, *Crassostrea virginica* (Gmelin), to infection by the protistan parasite, MSX. *Am. Malacol. Union Bull.* **32**, 23–24. (abstract).
- Farley, C. A. (1967). A proposed life cycle of *Minchinia nelsoni* (Haplosporida, Haplosporidiidae) in the American oyster *Crassostrea virginica*. *J. Protozool.* **14**, 616–625.
- Farley, C. A. (1968). *Minchinia nelsoni* (Haplosporida, Haplosporidiidae) disease syndrome in the American oyster, *Crassostrea virginica*. *J. Protozool.* **15**, 585–599.
- Farley, C. A. (1975). Epizootic and enzootic aspects of *Minchinia nelsoni* (Haplosporida) disease in Maryland oysters. *J. Protozool.* **22**, 418–427.
- Feng, S. Y. (1958). Observations on distribution and elimination of spores of *Nematopsis ostrearum* in oysters. *Proc. Natl. Shellfish. Assoc.* **48**, 162–173.
- Feng, S. Y., and Stauber, L. A. (1968). Experimental hexamitiasis in the oyster *Crassostrea virginica*. *J. Invertebr. Pathol.* **10**, 94–110.
- Field, I. A. (1923). Biology and economic value of the sea mussel *Mytilus edulis*. *Bull. Bur. Fish., Washington* (1921–22) **38**, 127–259.
- Ford, S. E. (1985a). Chronic infections of *Haplosporidium nelsoni* (MSX) in the oyster *Crassostrea virginica*. *J. Invertebr. Pathol.* **45**, 94–107.
- Ford, S. E. (1985b). Effects of salinity on survival of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber, and Mackin) in oysters. *J. Shellfish Res.* **5**, 85–90.
- Ford, S. E. (1986). Comparison of hemolymph proteins between resistant and susceptible oysters, *Crassostrea virginica*, exposed to the parasite *Haplosporidium nelsoni* (MSX). *J. Invertebr. Pathol.* **47**, 283–294.
- Ford, S. E. (1988). Host–parasite interactions in eastern oysters selected for resistance to *Haplosporidium nelsoni* (MSX) disease: Survival mechanisms against a natural pathogen. *Am. Fish. Soc., Spec. Publ.* **18**, 206–224.
- Ford, S. E., and Figueras, A. J. (1988). Effects of sublethal infection by the parasite *Haplosporidium nelsoni* (MSX) on gametogenesis, spawning, and sex ratios of oysters in Delaware Bay, USA. *Dis. Aquat. Org.* **4**, 121–133.
- Ford, S. E., and Haskin, H. H. (1982). History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957–1980. *J. Invertebr. Pathol.* **40**, 118–141.
- Ford, S. E., and Haskin, H. H. (1987). Infection and mortality patterns in strains of oysters *Crassostrea virginica* selected for resistance to the parasite *Haplosporidium nelsoni* (MSX). *J. Parasitol.* **73**, 368–376.
- Franc, A. (1980). Sur quelques aspects inédits du cycle de *Marteilia refringens* Grizel et coll., 1974, parasite de l'huître plate *Ostrea edulis* L. *Cah. Biol. Mar.* **21**, 99–106.
- Franc, A., and Arvy, L. (1969). Sur *Thanatostrea polymorpha* n. g., n. sp., agent de destruction des branchies et des palpes de l'huître portugaise. *C. R. Hebd. Séances Acad. Sci., Ser. D* **158**, 3189–3190.
- Franc, A., and Arvy, L. (1970). Données sur l'évolution de la "maladie des branchies" chez les huîtres et sur son agent causal: *Thanatostrea polymorpha* Franc et Arvy, 1969. *Bull. Biol. Fr. Belg.* **104**, 2–19.

- Goggin, C. L., and Lester, R. J. G. (1987). Occurrence of *Perkinsus* species (Protozoa: Apicomplexa) in bivalves from the Great Barrier Reef. *Dis. Aquat. Org.* **3**, 113–117.
- Grizel, H. (1976). Développement et cycle du parasite responsable de l'épizootie de l'huître plate *Ostrea edulis* L. *Haliotis* **5**, 61–67.
- Grizel, H. (1979). *Marteilia refringens* and oyster disease—Recent observations. *Mar. Fish. Rev.* **41**(1–2), 38–49.
- Grizel, H. (1985). "Étude des récentes épizooties de l'huître plate *Ostrea edulis* Linné et de leur impact sur l'ostréiculture bretonne," Ph. D. thesis, 145 pp. Acad. Montpellier, Montpellier, France.
- Grizel, H. and Tige, G. (1979). Observations sur le cycle de *Marteilia refringens*. *Haliotis* **8**, 327–330.
- Grizel, H. and Tige, G. (1982). Evolution of the hemocytic disease caused by *Bonamia ostreae*. *Proc. Int. Colloq. Invertebr. Pathol.*, 3rd pp. 258–259.
- Grizel, H., Comps, M., Bonami, J.-R., Cousserans, F., Duthoit, J. -L., and Le Pennec, M. A. (1974a). Recherche sur l'agent de la maladie de la glande digestive de *Ostrea edulis* Linné. *Sci. Pêche* **240**, 7–30.
- Grizel, H., Comps, M., Cousserans, F., Bonami, J.-R., and Vago, C. (1974b). Pathologie des invertébrés. Étude d'un parasite de la glande digestive observé au cours de l'épizootie actuelle de l'huître plate. *C. R. Hebd. Séances Acad. Sci., Ser. D* **279**, 783–784.
- Gurley, R. R. (1893). On the classification of the Myxosporidia, a group of protozoan parasites infesting fishes. *Bull. U.S. Fish. Comm.* **11**, 407–420.
- Gutiérrez, M. (1977a). Nota sobre marteiliasis en el mejillón, *Mytilus edulis* (L.), de la costa Noroeste de España. *Invest. Pesq.* **41**, 637–642.
- Gutiérrez, M. (1977b). Técnica de coloración del agente de la enfermedad de la glándula digestiva de la ostra plana, *Ostrea edulis* L. *Invest. Pesq.* **41**, 643–645.
- Haskin, H. H. (1961). Delaware Bay oyster mortalities. *Proc. Annu. Sess. Gulf Carib. Fish. Inst.* **13**, 109 (abstract).
- Haskin, H. H. (1976). Epizootiology of *Minchinia nelsoni* in oysters. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st pp. 166–168.
- Haskin, H. H., and Ford, S. E. (1979). Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. *Mar. Fish. Rev.* **41**, 54–63.
- Haskin, H. H., and Ford, S. E. (1982). *Haplosporidium nelsoni* (MSX) on Delaware Bay seed oyster beds: A host-parasite relationship along a salinity gradient. *J. Invertebr. Pathol.* **40**, 388–405.
- Haskin, H. H., and Ford, S. E. (1983). Quantitative effects of MSX disease (*Haplosporidium nelsoni*) on production of the New Jersey oyster beds in Delaware Bay, U.S.A. *Int. Counc. Explor. Sea, Doc. C.M.1983/Gen:7*, 21 pp. (minisymposium).
- Haskin, H. H., and Ford, S. E. (1987). Breeding for disease resistance in molluscs, In "Proceedings of the World Symposium on Selection, Hybridization, and Genetic Engineering in Aquaculture (1986)" (K. Tiews, ed.), Vol. 2, pp. 431–441. Bordeaux, France.
- Haskin, H. H., Canzonier, W. J., and Myhre, J. L. (1965). The history of "MSX" on Delaware Bay oyster grounds, 1957–1965. *Am. Malacol. Union Bull.* **32**, 20–21 (abstract).
- Haskin, H. H., Stauber, L. A., and Mackin, J. G. (1966). *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae) causative agent of the Delaware Bay oyster epizootic. *Science* **153**, 1414–1416.
- Hatt, P. (1928). L'évolution de la grégarine du homard (*Porospora gigantea* E. V. Bened.) chez les mollusques. *C. R. Soc. Biol.* **98**, 647–649.
- Hatt, P. (1931). L'évolution des porosporides chez les mollusques. *Arch. Zool. Exp. Gen.* **72**, 341–415.

- Hazard, E. I., and Oldacre, S. W. (1975). Revision of Microsporida (Protozoa) close to *Thelohania*, with descriptions of one new family, eight new genera, and thirteen new species. *U.S. Dep. Agric., Agric. Res. Serv., Tech. Bull.* **1530**, 104 pp.
- Henneguy, G., and Thélohan, P. (1892). Sur une sporozoaire parasite des muscles des crustacés décapodes. *C. R. Soc. Biol.* **4**, 585–588.
- Herrbach, B. (1971). Sur une affection parasitaire de la glande digestive de l'huître plate, *Ostrea edulis* Linné. *Rev. Trav. Inst. Pêches Marit.* **35**, 79–87.
- Hewatt, W. G., and Andrews, J. D. (1954). Oyster mortality studies in Virginia. I. Mortalities of oysters in trays at Gloucester Point, York River. *Tex. J. Sci.* **6**, 121–133.
- Hewatt, W. G., and Andrews, J. D. (1956). Temperature control experiments on the fungus disease, *Dermocystidium marinum*, of oysters. *Proc. Natl. Shellfish. Assoc.* **46**, 129–133.
- Hibbits, J., and Sparks, A. K. (1983). Observations on the histopathology caused by a parasitic ciliate (*Paranophrys* sp?) in the isopod *Gnorimosphaeroma oregonensis*. *J. Invertebr. Pathol.* **41**, 51–56.
- Hillman, R. E. (1978). The occurrence of *Minchinia* sp. (Haplosporida, Haplosporidiidae) in species of the molluscan borer, *Teredo*, from Barnegat Bay, New Jersey. *J. Invertebr. Pathol.* **31**, 265–266.
- Hillman, R. E. (1979). Occurrence of *Minchinia* sp. in species of the molluscan borer, *Teredo*. *Mar. Fish. Rev.* **41**(1–2), 21–24.
- Hillman, R. E. (1980). Life cycle stages of *Minchinia* sp. in *Teredo navalis*. *Am. Zool.* **20**, 961.
- Hillman, R. E., Maciolek, N. J., Lahey, J. I., and Belmore, C. I. (1982). Effects of a haplosporidan parasite, *Haplosporidium* sp., on species of the molluscan woodborer *Teredo* in Barnegat Bay, New Jersey. *J. Invertebr. Pathol.* **40**, 307–319.
- Hoese, H. D. (1963). Absence of *Dermocystidium marinum* at Port Aransas, Texas, with notes on an apparent inhibitor. *Tex. J. Sci.* **15**, 98–103.
- Hoese, H. D. (1964). Studies on oyster scavengers and their relation to the fungus *Dermocystidium marinum*. *Proc. Natl. Shellfish. Assoc.* **53**, 161–174.
- Hutton, R. F., Sogandares-Bernal, F., Eldred, B., Ingle, R. M., and Woodburn, K. D. (1959). Investigations on the parasites and diseases of saltwater shrimps (Penaeidae) of sports and commercial importance to Florida [preliminary report]. *Fla., State Board Conserv., Tech. Ser.* **26**, 1–38.
- Imai, T., Mori, K., Sugawara, Y., Tamate, H., Oizumi, J., and Itikawa, O. (1968). Studies on the mass mortality of oysters in Matsushima Bay. VII. Pathogenetic investigation. *Tohoku J. Agric. Res.* **19**(4), 250–264.
- Imamura, T. (1940). Two species of *Lagenophrys* from Sapporo. *Annot. Zool. Jpn.* **19**, 267–270.
- Iversen, E. S., and Manning, R. B. (1959). A new microsporidian parasite from the pink shrimp (*Penaeus duorarum*). *Trans. Am. Fish. Soc.* **88**, 130–132.
- Iversen, E. S., and Van Meter, N. N. (1964). A record of the microsporidian, *Thelohania duorara*, parasitizing the shrimp *Penaeus brasiliensis*. *Bull. Mar. Sci. Gulf Caribb.* **14**, 549–553.
- Johnson, C. A., and Bradbury, P. C. (1976). Observations on the occurrence of the parasitic ciliate *Synophrya* in decapods in coastal waters off the southeastern United States. *J. Protozool.* **23**, 252–256.
- Johnson, P. T. (1977). Paramoebiasis in the blue crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* **29**, 308–320.
- Johnston, L. B., Vernick, S. H., and Sprague, V. (1978). Light and electron microscope study of a new species of *Thelohania* (Microsporida) in the shrimp, *Pandalus jordani*. *J. Invertebr. Pathol.* **32**, 278–290.
- Katkansky, S. C., and Warner, R. W. (1970). Sporulation of a haplosporidan in a Pacific

- oyster (*Crassostrea gigas*) in Humboldt Bay, California. *J. Fish. Res. Board Can.* **27**, 1320–1321.
- Kelly, J. F. (1979). Tissue specificities of *Thelohania duorara*, *Agmasoma penaei*, and *Pleistophora* sp., microsporidian parasites of pink shrimp, *Penaeus duorarum*. *J. Invertebr. Pathol.* **33**, 331–339.
- Kent, M. L., Elston, R. A., Nerad, T. A., and Sawyer, T. K. (1987). An *Isonema*-like flagellate (Protozoa: Mastigophora) infection in larval geoduck clams, *Panope abrupta*. *J. Invertebr. Pathol.* **50**, 221–229.
- Kern, F. G. (1976). Sporulation of *Minchinia* sp. (Haplosporida, Haplosporidiidae) in the Pacific oyster *Crassostrea gigas* (Thunberg) from the Republic of Korea. *J. Protozool.* **23**, 498–500.
- Kern, F. G., Sullivan, L. C., and Takata, M. (1973). *Labyrinthomyxa*-like organisms associated with mass mortalities of oysters, *Crassostrea virginica*, from Hawaii. *Proc. Natl. Shellfish. Assoc.* **63**, 43–46.
- Kruse, D. N. (1959a). Parasites of commercial shrimp. *Bull. Assoc. S.E. Biol.* **6**(1), 28.
- Kruse, D. N. (1959b). "A Study of the Taxonomy, Morphology, Incidence and Biology of the Parasites of the Commercial Shrimp, *Penaeus aztecus* Ives, *P. duorarum* Burkenroad and *P. setiferus* (Linnaeus)," M.A. thesis. Florida State University, Tallahassee, Florida.
- Kruse, D. N. (1959c). Parasites of the commercial shrimps, *Penaeus aztecus* Ives, *P. duorarum* Burkenroad and *P. setiferus* (Linnaeus). *Tulane Stud. Zool.* **7**, 123–144.
- Kruse, D. N. (1966). Life cycle studies on *Nematopsis duorari* n. sp. (Gregarina, Porosporidae) a parasite of the pink shrimp (*Penaeus duorarum*) and pelecypod molluscs. *Diss. Abstr. B* **27**, 2919-B.
- Landau, H., and Galtsoff, P. S. (1951). Distribution of *Nematopsis* infection on the oyster grounds of the Chesapeake Bay and in other waters of the Atlantic and Gulf States. *Tex. J. Sci.* **3**, 115–130.
- Lauckner, G. (1983). Diseases of Mollusca: Bivalvia, In "Diseases of Marine Animals" (O. Kinne, ed.), Vol. II, pp.477–961. Biol. Anstalt. Helgol., Hamburg, FRG.
- Léger, L. (1897). Sur la présence des coccidies chez les mollusques lamellibranches. *C. R. Soc. Biol.* **49**, 987–988.
- Léger, L., and Duboscq, O. (1915). *Porospora nephropis* n. sp. *C. R. Soc. Biol.* **78**, 368–371.
- Léger, L., and Hollande, A. C. (1917). Sur un nouveau protiste a facies de *Chytridiopsis*, parasite des ovules de l'huître. *C. R. Soc. Biol.* **80**, 61–64.
- Lester, R. J. G. (1986). Abalone die-back caused by protozoan parasite? *Aust. Fish.* **45**(6), 26–27.
- Lester, R. J. G., and Davis, G. H. G. (1981). A new *Perkinsus* species (Apicomplexa, Perkinsea) from the abalone *Haliotis ruber*. *J. Invertebr. Pathol.* **37**, 181–187.
- Levine, N. D. (1970). Taxonomy of the Sporozoa. *J. Parasitol.* **56**, 208–209.
- Levine, N. D. (1971). Uniform terminology for the protozoan subphylum Apicomplexa. *J. Protozool.* **18**, 352–355.
- Levine, N. D. (1978). *Perkinsus* gen. n. and other new taxa in the protozoan phylum Apicomplexa. *J. Parasitol.* **64**, 549.
- Levine, N. D., Corliss, J. O., Cox, F. E. G., Deroux, G., Grain, J., Honigberg, B. M., Leedale, G. F., Loeblich, A. R., Lom, J., Lynn, D., Merinfeld, E. G., Page, F. C., Poljansky, G., Sprague, V., Vavra, J., and Wallace, F. G. (1980). A newly revised classification of the Protozoa. *J. Protozool.* **27**, 37–58.
- Li, M. F. (1976). A microparasite, the possible causative agent of Malpeque disease of oysters. *Proc. Can. Fed. Biol. Soc.* **19**, 158.
- Li, M. F. and Clyburne, S. (1979). Mortalities of blue mussel (*Mytilus edulis*) in Prince Edward Island. *J. Invertebr. Pathol.* **33**, 108–110.

- Lightner, D. V. (1975). Some potentially serious disease problems in the culture of penaeid shrimp in North America. *Proc. U.S.-Jpn. Meet. Aquacult.*, 3rd, pp. 75-97.
- Little, E. J., and Quick, J. A. (1976). Ecology, resource rehabilitation, and fungal parasitology of commercial oysters, *Crassostrea virginica* (Gmelin), in Pensacola estuary, Florida. *Fla. Mar. Res. Publ.* **21**, 1-85.
- Mackin, J. G. (1951). Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, and Collier. *Bull. Mar. Sci. Gulf Caribb.* **1**, 72-87.
- Mackin, J. G. (1953). Incidence of infection of oysters by *Dermocystidium* in the Barataria Bay area of Louisiana. *Conv. Addresses Natl. Shellfish. Assoc.* 1951 pp. 22-35.
- Mackin, J. G. (1956). *Dermocystidium marinum* and salinity. *Proc. Natl. Shellfish. Assoc.* **46**, 116-128.
- Mackin, J. G. (1960). Status of researches on oyster diseases in North America. *Proc. Annu. Sess. Gulf Caribb. Fish. Inst.* **13**, 98-113.
- Mackin, J. G. (1961). Mortalities of oysters. *Proc. Natl. Shellfish. Assoc.* **51**, 21-40.
- Mackin, J. G. (1962). Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publ. Inst. Mar. Sci., Univ. Tex.* **7**, 132-229.
- Mackin, J. G., and Boswell, J. L. (1956). The life cycle and relationships of *Dermocystidium marinum*. *Proc. Natl. Shellfish. Assoc.* **46**, 112-115.
- Mackin, J. G., and Ray, S. M. (1966). The taxonomic relationships of *Dermocystidium marinum*, Mackin, Owen, and Collier. *J. Invertebr. Pathol.* **8**, 544-545.
- Mackin, J. G., Owen, H. M., and Collier, A. (1950). Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp., in *Crassostrea virginica* (Gmelin). *Science*, **111**, 328-329.
- Mackin, J. G., Korringa, P., and Hopkins, S. H. (1952). Hexamitiasis of *Ostrea edulis* L. and *Crassostrea virginica* (Gmelin). *Bull. Mar. Sci. Gulf Caribb.* **1**, 266-277.
- MacLean, S. A., and Ruddell, C. L. (1978). Three new crustacean hosts for the parasitic dinoflagellate *Hematodinium perezii* (Dinoflagellata: Syndinidae). *J. Parasitol.* **64**, 158-160.
- Masso-Bolivar, J. M. (1978). La enfermedad de la glándula digestiva de la ostra (*Ostrea edulis* L.) en las rías bajas. *Bol. Inst. Esp. Oceanogr.* **4**, 125-140.
- Matsusato, T., Hoshina, T., Arakawa, K., and Masumura, K. (1977). Studies on the so-called abnormal egg-mass of Japanese oyster, *Crassostrea gigas* (Thunberg). I. *Hiroshima-ken Suisan Shikenjo Jikyo Hokoku* **8**, 9-25.
- Menzel, R. W., and Hopkins, S. H. (1955). The growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus*. *J. Parasitol.* **41**, 333-342.
- Meyers, T. R., Koeneman, T. M., Botelho, C., and Short, S. (1987). Bitter crab disease: A fatal dinoflagellate infection and marketing problem for Alaskan tanner crabs *Chionoecetes bairdi*. *Dis. Aquat. Org.* **3**, 195-216.
- Mialhe, E., Bachere, E., Le Bec, C., and Grizel, H. (1985). Isolement et purification de *Marteilia* (Protozoa; Asctospora) parasites de bivalves marins. *C. R. Hebd. Séances Acad. Sci.* **301**, 137-141.
- Mix, M. C., and Sprague, V. (1974). Occurrence of a haplosporidian in native oysters (*Ostrea lurida*) from Yaquina Bay and Alsea Bay, Oregon. *J. Invertebr. Pathol.* **23**, 252-254.
- Montreuil, P. (1954). Parasitological investigations. *Rapp. Annu. Stn. Biol. Mar. Dep. Pêch.*, 1953 **50** (Appendix 5), 69-73.
- Morado, J. F., Sparks, A. K., and Reed, S. K. (1984). A coccidian infection of the kidney of the native littleneck clam, *Protothaca staminea*. *J. Invertebr. Pathol.* **43**, 207-217.
- Morel, M., and Tige, G. (1974). Maladie de la glande digestive de l'huître plate. *Bull. Inst. Pêch. Marit. Maroc.* **241**, 33-40.

- Newman, M. W., and Johnson, C. A., III (1976). A disease of blue crabs (*Callinectes sapidus*) caused by parasitic dinoflagellates, *Hematodinium* sp. *J. Parasitol.* **6**, 554–555.
- Newman, M. W., and Ward, G. W., Jr. (1973). An epizootic of blue crabs, *Callinectes sapidus*, caused by *Paramoeba pernicio*sa. *J. Invertebr. Pathol.* **22**, 329–334.
- Newman, M. W., Johnson, C. A., III, and Pauley, G. B. (1976). A Minchinia-like haplosporidan parasitizing blue crabs, *Callinectes sapidus*. *J. Invertebr. Pathol.* **27**, 311–315.
- Ogle, J., and Flurry, K. (1980). Occurrence and seasonality of *Perkinsus marinus* (Protozoa: Apicomplexa) in Mississippi oysters. *Gulf Res. Rep.* **6**, 423–425.
- Olson, R. E., and Lannan, C. N. (1984). Prevalence of microsporidian infection in commercially-caught pink shrimp, *Pandalus jordani*. *J. Invertebr. Pathol.* **43**, 407–413.
- Overstreet, R. M. (1973). Parasites of some penaeid shrimps with emphasis on reared hosts. *Aquaculture* **2**, 105–140.
- Overstreet, R. M. (1977). Microsporidiosis of the blue crab, In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann, ed.), pp. 117–125. Elsevier, Amsterdam.
- Overstreet, R. M. (1978). Marine maladies? Worm, germs and other symbionts from the northern Gulf of Mexico. *Miss.-Ala. Sea Grant Consortium Publ.* **78-021**, 140 pp.
- Overstreet, R. M., and Whatley, E. C. (1975). Prevention of microsporidiosis in the blue crab, with notes on natural infections. *Proc. Annu. Meet.—World Maric. Soc.* **6**, 335–345.
- Owen, H. M., Walters, L. L., and Bregan, L. A. (1952). Etiological studies on oyster mortality. I. *Nematopsis ostrearum* Prytherch 1940 (Sporozoa: Porosporidae). *J. Mar. Res.* **10**, 82–90.
- Parsons, D. G., and Khan, R. A. (1986). Microsporidiosis in the northern shrimp, *Pandalus borealis*. *J. Invertebr. Pathol.* **47**, 74–81.
- Pauley, G. B., Newman, M. W., and Gould, E. (1975). Serum changes in the blue crab, *Callinectes sapidus*, associated with *Paramoeba pernicio*sa, the causative agent of gray crab disease. *Mar. Fish. Rev.* **37**(5–6), 34–38.
- Pérez, C. (1904). Sur une microsporidie parasite du *Carcinus maenas*. *C. R. Soc. Biol.* **57**, 214–215.
- Pérez, C. (1905a). Microsporidies parasites des crabes d'Arcachon. Note préliminaire. *Trav. Lab. Soc. Sci. Arcachon* **8**, 15–36.
- Pérez, C. (1905b). Sur une nouvelle glugéidée parasite du *Carcinus maenas*. *C. R. Soc. Biol.* **58**, 146–151.
- Pérez, C. (1907). Sur un cas d'envahissement de l'ovaire par *Thelohania maenadis*. *C. R. Soc. Biol.* **60**, 1091–1092.
- Perkins, F. O. (1969). Ultrastructure of vegetative stages in *Labyrinthomyxa marina* (*Dermocystidium marinum*), a commercially significant oyster pathogen. *J. Invertebr. Pathol.* **13**, 199–222.
- Perkins, F. O. (1976a). *Dermocystidium marinum* infection in oysters. *Mar. Fish. Rev.* **38**(10), 19–21.
- Perkins, F. O. (1976b). Ultrastructure of sporulation in the European flat oyster pathogen, *Marteilia refringens*—taxonomic implications. *J. Protozool.* **23**, 64–74.
- Perkins, F. O., and Menzel, R. W. (1966). Morphological and cultural studies of a motile stage in the life cycle of *Dermocystidium marinum*. *Proc. Natl. Shellfish. Assoc.* **56**, 23–30.
- Perkins, F. O., and Menzel, R. W. (1967). Ultrastructure of sporulation in the oyster pathogen *Dermocystidium marinum*. *J. Invertebr. Pathol.* **9**, 205–229.
- Perkins, F. O., and Wolf, P. H. (1976). Fine structure of *Marteilia sydneyi* sp. n.—haplosporidian pathogen of Australian oysters. *J. Parasitol.* **62**, 528–538.
- Pichot, Y. (1986). Sporulation d'*Haplosporidium* sp. (Haplosporida, Haplosporidiidae) chez l'huître *Ostrea edulis* L. du Bassin d'Arcachon (France), *Eur. Aquacult. Soc., Spec. Publ.* **9**, 119–126.

- Pichot, Y., Comps, M., and Deltreil, J. P. (1979). Recherches sur *Haplosporidium* sp. (Haplosporida, Haplosporidiidae) parasite de l'huître plate *Ostrea edulis* L. *Rev. Trav. Inst. Pêches Marit.* **43**(4), 405–408.
- Pichot, Y., Comps, M., Tige, G., and Rabouin, A. (1980). Recherches sur *Bonamia ostreae* gen. n., sp. n. parasite nouveau de l'huître plate *Ostrea edulis* L. *Rev. Trav. Inst. Pêches Marit.* **43**, 131–140.
- Poder, M., Cahour, A., and Balouet, G. (1982). Hemocytic parasitosis in European oyster *Ostrea edulis* L.: Pathology and contamination, *Proc. Int. Colloq. Invert. Pathol.*, 3rd pp. 254–257.
- Poisson, R. (1929). À propos de l'*Anophrys maggii* Cattaneo, infusoire parasite du sang du *Carcinus maenas* L. (Crustacé Décapode); sur son identité avec l'*Anophrys sarcophaga* Coh. *C. R. Soc. Biol. Fr. Belg.* **64**, 288–331.
- Poisson, R. (1930). Observations sur *Anophrys sarcophaga* Cohn (= *A. maggii* Cattaneo) infusoire holotriche marin et sur son parasitisme possible chez certains crustacés. *Bull. Biol. Fr. Belg.* **64**, 288–331.
- Prytherch, H. F. (1938). Life-cycle of a sporozoan parasite of the oyster. *Science* **88**, 451–452.
- Prytherch, H. F. (1940). The life history and morphology of *Nematopsis ostrearum*, sp. nov., a gregarine parasite of the mud crab and oyster. *J. Morphol.* **66**, 39–65.
- Quick, J. A., and Mackin, J. G. (1971). Oyster parasitism by *Labyrinthomyxa marina* in Florida. *Prof. Pap. Ser., Fla., Dep. Nat. Resour.* **13**, 1–55.
- Ray, S. M. (1952). A culture technique for diagnosis of infections with *Dermocystidium marinum* Mackin, Owen, and Collier in oysters. *Science* **116**, 360–361.
- Ray, S. M. (1954a). Experimental studies on the transmission and pathogenicity of *Dermocystidium marinum*, a fungus disease of oysters. *J. Parasitol.* **40**, 235.
- Ray, S. M. (1954b). Biological studies of *Dermocystidium marinum*, a fungus parasite of oysters. *Rice Inst. Pam., Spec. Issue, Monogr. Biol.* 114 pp.
- Ray, S. M. (1954c). Studies on the occurrence of *Dermocystidium marinum* in young oysters. *Conv. Addresses Natl. Shellfish. Assoc.* 1953 pp. 80–92.
- Ray, S. M. (1966a). Notes on the occurrence of *Dermocystidium marinum* on the Gulf of Mexico coast during 1961 and 1962. *Proc. Natl. Shellfish. Assoc.* **54**, 45–54.
- Ray, S. M. (1966b). A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proc. Natl. Shellfish. Assoc.* **54**, 55–69.
- Ray, S. M., and Chandler, A. C. (1955). *Dermocystidium marinum*, a parasite of oysters. *Exp. Parasitol.* **4**, 172–200.
- Ray, S. M., and Mackin, J. G. (1955). Studies of pathogenesis of *Dermocystidium marinum*. *Proc. Natl. Shellfish. Assoc.* **45**, 164–167.
- Ray, S. M., Mackin, J. G., and Boswell, J. L. (1953). Quantitative measurement of the effect on oysters of disease caused by *Dermocystidium marinum*. *Bull. Mar. Sci. Gulf Caribb.* **3**, 6–33.
- Rosenfield, A. (1964). Studies of oyster microparasites. *U.S. Fish Wildl. Serv., Circ.* **200**, 30–37.
- Rosenfield, A., and Sindermann, C. J. (1966). The distribution of "MSX" in middle Chesapeake Bay. *Proc. Natl. Shellfish. Assoc.* **56**, 6 (abstract).
- Sawyer, T. K. (1969). Preliminary study on the epizootiology and host-parasite relationship of *Paramoeba* sp. in the blue crab, *Callinectes sapidus*. *Proc. Natl. Shellfish. Assoc.* **59**, 60–64.
- Sawyer, T. K., and MacLean, S. A. (1978). Some protozoan diseases of decapod crustaceans. *Mar. Fish. Rev.* **40**(10), 32–35.
- Scheltema, R. S. (1962). The relationship between the flagellate protozoon *Hexamita* and the oyster *Crassostrea virginica*. *J. Parasitol.* **48**, 137–141.

- Schlicht, F. G., and Mackin, J. G. (1968). *Hexamita nelsoni* sp. n. (Poly-mastigina: Hexamitidae) parasitic in oysters. *J. Invertebr. Pathol.* **11**, 35–39.
- Sims, H. W. (1966). An annotated bibliography of the spiny lobsters, families Palinuridae and Scyllaridae. *Fla. State Board Conserv., Tech. Ser.* **48**, 1–84.
- Sindermann, C. J. (1966). Parasites of oysters, *Crassostrea virginica*, from the east coast of North America, In "Proceedings of the First International Congress of Parasitology" (A. Corradetti, ed.), Vol. I, pp. 585–586. Pergamon, Oxford, England.
- Sindermann, C. J., and Rosenfield, A. (1967). Principal diseases of commercially important marine bivalve Mollusca and Crustacea. *U.S. Fish Wildl. Serv., Fish. Bull.* **66**, 335–385.
- Sogandares-Bernal, F. (1962). Presumable microsporidiosis in the dwarf crayfishes *Cambarus puer* Hobbs and *C. shufeldti* (Faxon) in Louisiana. *J. Parasitol.* **48**, 493.
- Sparks, A. K. (1985). "Synopsis of Invertebrate Pathology Exclusive of Insects," 423 pp. Elsevier, Amsterdam.
- Sparks, A. K., Hibbits, J., and Fegley, J. C. (1982). Observations on the histopathology of a systemic ciliate (*Paranophrys* sp.?) disease in the Dungeness crab, *Cancer magister*. *J. Invertebr. Pathol.* **39**, 219–228.
- Sprague, V. (1949). Species of *Nematopsis* in *Ostrea virginica*. *J. Parasitol.* **35**, (Suppl. 42) (abstract).
- Sprague, V. (1950a). Notes on three microsporidian parasites of decapod Crustacea of Louisiana coastal waters. *Occas. Pap. Marine Lab., Louisiana State Univ.* **5**, 1–8.
- Sprague, V. (1950b). *Thelohania cambari* n. sp., a microsporidian parasite of North American crayfish. *J. Parasitol.* **36**, 46 (abstract).
- Sprague, V. (1954). Protozoa. *Bull. U.S. Bur. Fish.* **55**, 243–256.
- Sprague, V. (1963a). *Minchinia louisiana* n. sp. (Haplosporidia, Haplosporidiidae), a parasite of *Panopeus herbstii*. *J. Protozool.* **10**, 267–274.
- Sprague, V. (1963b). Revision of genus *Haplosporidium* and restoration of genus *Minchinia* (Haplosporidia, Haplosporidiidae). *J. Protozool.* **10**, 263–266.
- Sprague, V. (1965a). *Nosema* sp. (Microsporidia, Nosematidae) in the musculature of the crab *Callinectes sapidus*. *J. Protozool.* **12**, 66–70.
- Sprague, V. (1965b). Observations on *Chytridiopsis mytilovum* (Field), formerly *Haplosporidium mytilovum* Field (Microsporidia?). *J. Protozool.* **12**, 385–389.
- Sprague, V. (1966). Two new species of *Plistophora* (Microsporidia, Nosematidae) in decapods, with particular reference to one in the blue crab. *J. Protozool.* **13**, 196–199.
- Sprague, V. (1970a). Some protozoan parasites and hyperparasites in marine decapod Crustacea, In "A Symposium on Diseases of Fishes and Shellfishes" (S. F. Snieszko, ed.), Spec. Publ. 5, pp. 416–430. Am. Fish. Soc., Washington, D.C.
- Sprague, V. (1970b). Some protozoan parasites and hyperparasites in marine bivalve molluscs, In "A Symposium on Diseases of Fishes and Shellfishes" (S. F. Snieszko, ed.), Spec. Publ. 5, pp. 511–526. Am. Fish. Soc., Washington, D.C.
- Sprague, V. (1977). Systematics of the Microsporidia, In "Comparative Pathobiology" (L. A. Bulla and T. C. Cheng, eds.), Vol. 2, pp. 1–30. Plenum, New York.
- Sprague, V. (1978). Comments on trends in research on parasitic diseases of shellfish and fish. *Mar. Fish. Rev.* **40**, 26–30.
- Sprague, V., and Beckett, R. L. (1966). A disease of blue crabs (*Callinectes sapidus*) in Maryland and Virginia. *J. Invertebr. Pathol.* **8**, 287–289.
- Sprague, V., and Beckett, R. L. (1968). The nature of the etiological agent of gray crab disease. *J. Invertebr. Pathol.* **11**, 503.
- Sprague, V., and Orr, P. E., Jr. (1953). Studies on *Nematopsis*. III. *N. ostrearum* and *N. prytherchi* with special references to the host-parasite relations. *Conn. Addresses Natl. Shellfish. Assoc.* 1952 pp. 26–43.

- Sprague, V., and Orr, P. E. (1955). *Nematopsis ostrearum* and *N. prytherchi* (Eugregarinida, Porosporidae) with special reference to the host-parasite relations. *J. Parasitol.* **41**, 89–104.
- Sprague, V., Beckett, R. L., and Sawyer, T. K. (1969). A new species of *Paramoeba* (Amoebida, Paramoebidae) parasitic in the crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* **14**, 167–174.
- Stein, J. E., Denison, J. G., and Mackin, J. G. (1961). *Hexamita* sp. and an infectious disease in the commercial oyster *Ostrea lurida*. *Proc. Natl. Shellfish. Assoc.* **50**, 67–81.
- Taylor, R. L. (1966). *Haplosporidium tumefacientis* sp. n., the etiologic agent of a disease of the California sea mussel, *Mytilus californianus* Conrad. *J. Invertebr. Pathol.* **8**, 109–121.
- Théodorides, J. (1961). Sur la distinction entre les grégaires des familles des Cephaloidophoridae et des Porosporidae, parasites de crustacés décapodes. *C. R. Soc. Biol.* **252**, 3640–3642.
- Théodorides, J. (1962). Grégaires d'invertébrés marins de la région de Banyuls. I. Eugregarines parasites de crustacés décapodes. *Vie Milieu* **13**, 95–122.
- Tige, G., Grizel, H., and Comps, M. (1980). Données sur le nouveau parasite de l'huître plate. Situation épidémiologique. *Int. Counc. Explor. Sea, Spec. Meet. Dis. Commer. Important Mar. Fish and Shellfish Pap.* 39.
- Tuzet, O., and Ormieres, R. (1961). Sur quelques grégaires parasites de crustacés décapodes. *Ann. Sci. Nat., Zool. Biol. Anim.* **3**, 773–783.
- van Banning, P. (1977). *Minchinia armoricana* sp. nov. (Haplosporida), a parasite of the European flat oyster, *Ostrea edulis*. *J. Invertebr. Pathol.* **30**, 199–206.
- van Banning, P. (1979). Haplosporidian diseases of imported oysters, *Ostrea edulis*, in Dutch estuaries. *Mar. Fish. Rev.* **41**, (1–2), 8–18.
- van Banning, P. (1982). Some aspects of the occurrence, importance and control of the oyster pathogen *Bonamia* in a Dutch oyster culture. *Proc. Int. Colloq. Invertebr. Pathol.*, 3rd pp. 261–263.
- van Banning, P. (1985). Control of *Bonamia* in Dutch oyster culture, In "Fish and Shellfish Pathology" (A. E. Ellis, ed.), pp. 393–396. Academic Press, London.
- Vernick, S. H., and Sprague, V. (1970). *In vitro* muscle lysis accompanying treatment with extract of crab muscle infected with *Nosema* sp. *J. Parasitol.* **56**, 352–353 (abstract).
- Viosca, P., Jr. (1945). A critical analysis of practices in the management of warmwater fish with a view to greater food production. *Trans. Am. Fish. Soc.* **73**, 274–283.
- Vivares, C. P., and Cuq, J. L. (1981). Physiological and metabolic variations in *Carcinus mediterraneus* (Crustacea: Decapoda) parasitized by *Thelohania mediterraneus* (Microspora: Microsporida): An ecophysiopathological approach. *J. Invertebr. Pathol.* **37**, 38–46.
- Vivares, C. P., and Sprague, V. (1979). The fine structure of *Ameson pulvis* (Microspora, Microsporida) and its implications regarding classification and chromosome cycle. *J. Invertebr. Pathol.* **33**, 40–52.
- Vivares, C. P., Brehélin, M., Cousserans, F., and Bonami, J.-R. (1982). Mise en évidence d'une nouvelle Haplosporidie parasite de l'huître plate *Ostrea edulis* L. *C. R. Hebd. Séances Acad. Sci., Ser. III* **295**, 127–130.
- Watson, M. E. (1915). Some new gregarine parasites from Arthropoda. *J. Parasitol.* **2**, 27–36.
- Watson, M. E. (1916a). Three new gregarines from marine Crustacea. *J. Parasitol.* **2**, 129–136.
- Watson, M. E. (1916b). Observations on polycistid gregarines from Arthropoda. *J. Parasitol.* **3**, 65–75.
- Watson-Kamm, M. E. (1922). Studies on gregarines. II. III. *Biol. Monogr.* **7**, 1–104.
- Weidner, E. (1970). Ultrastructural study of microsporidian development. I. *Nosema* sp. Sprague, 1965 in *Callinectes sapidus* Rathbun. *Z. Zellforsch.* **105**, 33–54.

- White, M. E., Powell, E. N., Ray, S. M., and Wilson, E. A. (1987). Host-to-host transmission of *Perkinsus marinus* in oyster (*Crassostrea virginica*) populations by the ectoparasitic snail *Boonea impressa* (Pyramidellidae). *J. Shellfish. Res.* **6**, 1–5.
- Wolf, P. H. (1972). Occurrence of a haplosporidian in Sydney rock oysters (*Crassostrea commercialis*) from Moreton Bay, Queensland, Australia. *J. Invertebr. Pathol.* **19**, 416–417.
- Wolf, P. H. (1979). Life cycle and ecology of *Marteilia sydneyi* in the Australian oyster, *Crassostrea commercialis*. *Mar. Fish. Rev.* **41**(1–2), 70–72.
- Wood, J. L., and Andrews, J. D. (1962). *Haplosporidium costale* (Sporozoa) associated with a disease of Virginia oysters. *Science* **136**, 710–711.
- Woodburn, K. D., Eldred, B., Clark, E., Hutton, R. F., and Ingle, R. M. (1957). The live bait shrimp industry of the west coast of Florida (Cedar Key to Naples). *Fla. State Board Conserv., Tech. Ser.* **21**, 1–31.

6

Helminths

I. INTRODUCTION

Helminth parasites of molluscan and crustacean shellfish include the trematodes, cestodes, and nematodes (and to a lesser extent the acanthocephalans, parasitic turbellarians, nemerteans, and leeches). Since many of the helminths are large enough to be visible grossly, the descriptive literature is extensive, especially for larval trematodes in molluscs. The complex life cycles of trematodes have provided continuing challenges for parasitological detective work, and larval trematodes may have severe effects on molluscs, which serve as their first intermediate hosts—weakening them, sterilizing them, and causing behavioral changes which lessen survival potential. All of these factors, plus some public health concerns about trematodes infective to humans, have combined to encourage the production of a substantial corpus of literature. Our concern here, though, is principally with those helminth parasites which cause significant pathology in molluscan and crustacean hosts.

The larval trematodes clearly occupy center stage in this chapter, but other helminths—particularly the larval cestodes and nematodes—have received some scientific attention. Notable here are trypanorhynch cestodes such as *Tylocephalum* and *Echeneibothrium* from bivalve molluscs and the nematodes *Echinocephalus* from abalone and *Sulcascaris* from clams and scallops. Crustacea harbor a wider variety of helminths, but their overall effects on shrimp, crabs, and lobsters seem to be minimal, except in unusual cases of massive invasion.

The objective of this chapter is to provide an overview of worm parasites of shellfish, emphasizing those which are more than biological curiosities: those which cause disease.

II. DISEASES OF MOLLUSCS CAUSED BY HELMINTHS

Helminths—trematodes, cestodes, and nematodes—of molluscs can be debilitating to their hosts, causing poor growth and condition and re-

ducing fecundity. Of the various worm parasites, larval trematodes are clearly most destructive to molluscs; in some instances changes in host population abundance have been attributed to high levels of parasitization. In addition to these limited effects on abundance, helminth parasites of marine molluscs are noted as

- Being involved in pearl formation in mussels and other bivalves
- Potential biological control agents (trematodes of shell-boring snails), as a consequence of sterilizing the hosts
- Being hyperparasitized by protozoa—this hyperparasitization even occurring as an epizootic in one instance (a haplosporidan in nematodes parasitic in surf clams)
- Possible causes of digestive disturbances in humans, as a consequence of ingesting uncooked parasitized bivalves
- Etiological agents for human schistosome dermatitis (caused by larval avian schistosome cercariae from gastropods)

Larval trematodes have captured the attention of parasitologists for over a century, particularly because of their complex life histories, involving a succession of stages. Interspersed in the vast descriptive literature there are some reports of trematodes as causative agents of disease in molluscs of economic importance. These are the gems for which we are searching in this section on helminth diseases, even though some passing attention will be paid to a few cestodes and nematodes.

A. Trematodes

Trematode larvae are undoubtedly the most important helminth parasites of marine molluscs. To introduce a modicum of disorder into consideration of these larval trematode parasites, it should be pointed out early that they may use molluscs as first intermediate hosts (with sporocyst, redial, and cercarial stages) or as second intermediate hosts (metacercarial stage), recognizing that in some instances the mollusc may act as the host for both stages. Of the several ways that the almost overwhelming literature on larval trematodes of molluscs could be assembled, this summary is structured around host groups: oysters, mussels, clams, and snails. Much greater detail can be found in an exhaustive review of the subject by Lauckner (1983), encompassing 130 pages of text!

Members of the family Bucephalidae are parasitic in economic marine animals as sporocysts and cercariae in oysters and mussels, as metacercariae in small fish, and as adults in predatory fish. Principal damage occurs in bivalves in the form of sterilization and tissue destruction,

although metacercariae in fish flesh may reduce market value and increase processing costs. A number of studies have suggested that larval bucephalid trematodes are capable of debilitating and even killing their bivalve hosts (Deltreil and His, 1970; Lauckner, 1983).

European and American oysters are parasitized by larval trematodes of the genus *Bucephalus* (Fig. 23). *Bucephalus haimeanus* was first reported by Lacaze-Duthiers (1854) in European oysters in the Mediterranean Sea, and *Bucephalus cuculus* was described by McGrady (1874) in American oysters from South Carolina. Sporocysts occur in the gonads and the digestive gland of the oyster (Fig. 24) and sterilize the host. In advanced infections sporocysts spread to gills, mantle, and even the adductor muscle (Cheng and Burton, 1965). The tentative (and possibly equivocal) life cycle of the parasite, as proposed by Tennent (1906), included silverside minnows, *Menidia menidia*, as second intermediate hosts, and gars, *Tylosurus marinus*, as definitive hosts. Hopkins (1954a) found bucephalid metacercariae in striped mullet, *Mugil cephalus*, and white mullet, *Mugil curema*. He attempted to resolve some of the confusion about names of bucephalids and concluded that, in the absence of further experimental evidence, the correct name of the American oyster bucephalid was *B. cuculus*.

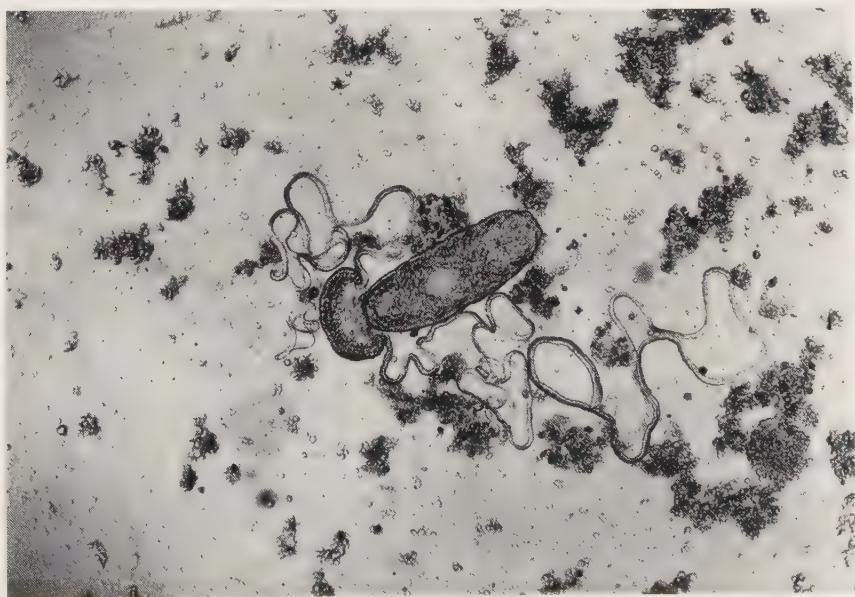


Figure 23. Cercaria of *Bucephalus* teased from oyster tissue.

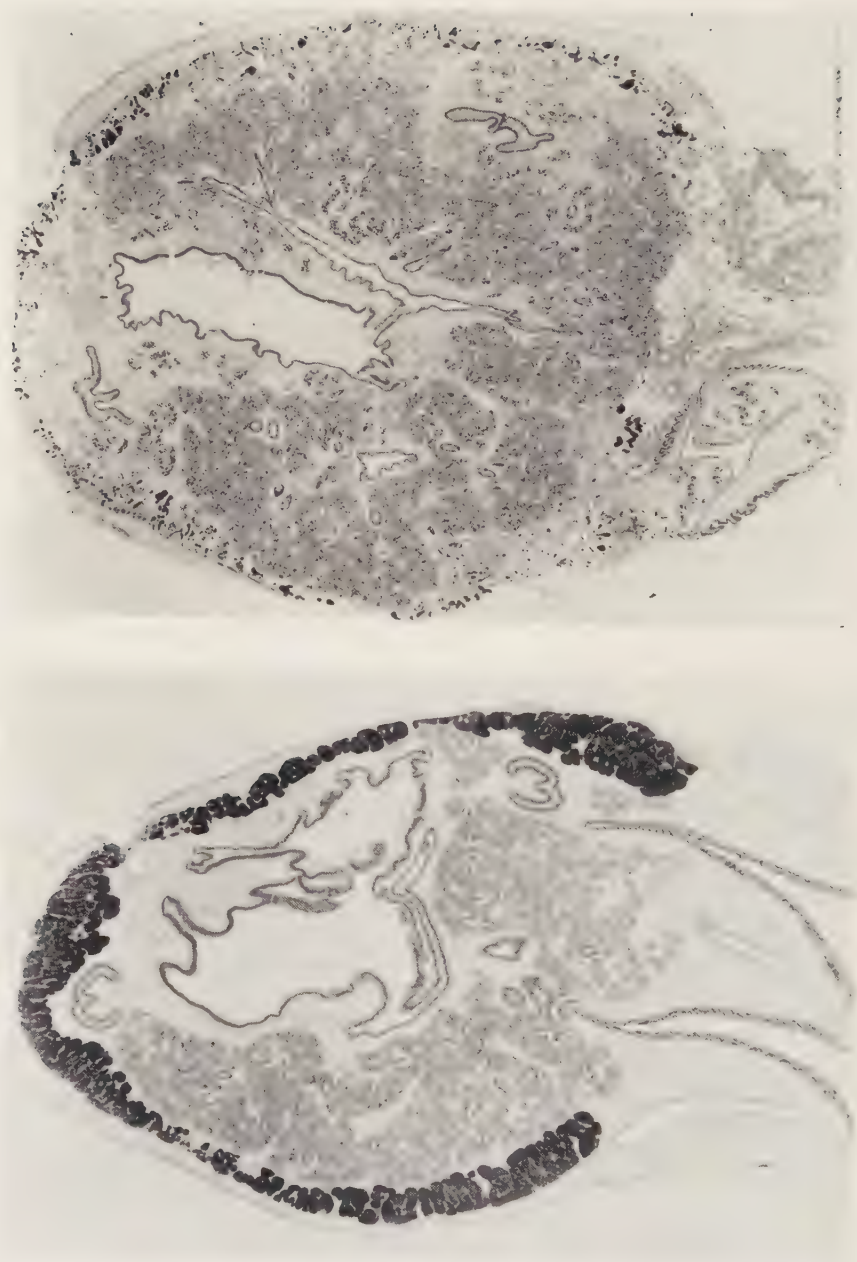


Figure 24. Cross sections of oysters (normal below) showing destruction of the darkly staining gonads by the larval trematode *Bucephalus*.

Hopkins (1957b) reported parasitization by *B. cuculus* in excess of 33% of the oysters in some localized areas of the U.S. coastline, although prevalences were generally lower than this, especially in open waters. Early infections temporarily stimulated growth of the host, but older infections retarded it (Menzel and Hopkins, 1955a,b). The interesting, if somewhat facetious, observation was made that *Bucephalus* might be considered a gastronomically beneficial parasite in southern waters, since infected oysters have an excellent flavor and are fat and glycogen rich throughout the year, whereas normal oysters are spawned out, thin, and relatively tasteless during part of the year. The story was spoiled by later histochemical studies in which it was pointed out that parasite-induced changes in fat metabolism resulted in buildup of butyric and other short-chain fatty acids, which may be toxic to humans (Cheng, 1965, 1967).

Larval trematodes of the family Bucephalidae occur in New Zealand oysters, *Ostrea lutaria*. Oysters imported from New Zealand and maintained for breeding studies in Scotland were frequently parasitized and the percentage of mortality was much higher among parasitized individuals than among normal ones (Millar, 1963). Howell (1966) experimentally completed most of the important features of the life cycle of *Bucephalus longicornutus* from *O. lutaria*. Cercariae encysted in fins, skin, and muscles of several genera of experimental intermediate hosts. Adult worms were recovered from the intestine of the scarpee, *Scorpaena cardinalis*, after experimental feedings with metacercariae. Adult *B. longicornutus* were also recovered from monkfish, *Kathetostoma giganteum*, from Cook Strait, New Zealand. In a later paper Howell (1967) suggested that an observed decline in the abundance of oysters on particular New Zealand beds was largely due to the high prevalence of *B. longicornutus*.

Trematodes of several families that infect marine bivalve molluscs may be hyperparasitized by haplosporidans (ascetosporans) or microsporeans. Hyperparasitization of sporocysts of *Bucephalus* in American oysters from the Gulf of Mexico was reported (Mackin and Loesch, 1955). Blackish discoloration of oyster mantle and viscera and destruction of sporocysts were followed by the release of hyperparasitic haplosporidan spores into host oyster tissue. A pronounced cellular reaction was elicited in the localized areas where spores were found in oyster tissue. The hyperparasite was not named, but it was considered on the basis of spore morphology to be a haplosporidan distinct from the parasite, *Urosporidium pelseeneeri*, found in sporocysts in clams of the genus *Donax*. Howell (1967) described *Urosporidium constantae* from *B. longicornutus* parasitizing *O. lutaria*. The haplosporidan completely destroyed embryonic cercariae within the sporocyst system. Howell proposed

(with reservation) that the hyperparasite might be used as a biological control measure in areas of high trematode prevalence.

A microsporean hyperparasite, *Nosema dollfusi*, of *B. cuculus* in *Crassostrea virginica* was described (Sprague, 1964), with the speculation that escape of the protozoan into the tissues of the oyster could contribute to death of the host—a speculation similar to that made about haplosporidan hyperparasites of oysters (Shuster and Hillman, 1963; Cheng, 1964).

Other larval trematodes also occur in oysters. Metacercariae of *Gymnophalloides tokiensis* encyst, often in great numbers, in the mantle and the gills of Pacific oysters, *Crassostrea gigas*, in Japan (Fujita, 1925, 1943; Ching, 1972). The host's physiology is disturbed, growth is halted, and reproduction is inhibited (Hoshina and Ogino, 1951). Marine birds are definitive hosts for the parasite. Metacercariae of *Proctoeces ostreae* are also found in Japanese oysters. About 10% of the oysters in Hiroshima Bay were infected by the larval trematode, which localizes in gonad tissue. European oysters harbor the related *Proctoeces maculatus*. Definitive hosts are labrid fishes in Europe, and snappers, *Pagrosomus major*, and red groupers, *Epinephelus akaara*, in Japan.

Massive invasion of American oysters from the southern tip of Texas by metacercariae was reported by Little *et al.* (1966). Feeding experiments showed that the trematodes were *Acanthoparyphium spinulosum*, which matures in the intestine of shore birds. Most of the oysters examined had metacercariae in the mantle, with an average of 45 worms per oyster.

Pearl-producing bivalves of the family Pteriidae, called "pearl oysters" but taxonomically remote from edible oysters of the family Ostreidae, occur in many parts of the world (Sivalingam, 1962). Interest in parasites of pearl oysters has naturally centered on the larval worms considered responsible for pearl formation (Jameson, 1902, 1912; Wright, 1966), but a few other parasites and diseases have been recognized. Pearl oysters of Japan, *Pinctada martensii*, are commonly infected with sporocysts and cercariae of a bucephalid trematode described as *Bucephalus margaritae* by Ozaki and Ishibashi (1934). Experimental infections of several species of small fish with cercariae from pearl oysters (Sakaguchi, 1962, 1966a) produced metacercariae identified as *Bucephalus varicus* (Manter, 1940). Adult trematodes were found in the digestive tracts of carangid fishes, *Caranx sexfasciatus* and *Caranx ignobilis*, which were abundant in the waters near oyster farms where pearl oysters were heavily infected (Sakaguchi, 1966b). The life cycle stages were completed when Sakaguchi (1967) obtained adult *B. varicus* from *C. sexfasciatus*, *C. ignobilis*, and *Caranx equula* which had been fed metacercariae grown in

the forage fish, *Rudarius ercodes*, by experimental infections with cercariae from pearl oysters. Marked decline in the condition of pearl oysters resulted from invasion by larval trematodes. Sporocysts were found to overwinter in the host, and production of cercariae began again when water temperatures rose in the spring (Sakaguchi, 1965). Pearl oysters infected during the preceding year suffered high mortalities after the insertion of pearl cores, and high percentages of pearls produced by infected individuals were of low quality (Sakaguchi, 1964, 1965, 1968).

Invasion of mussels by larval trematodes is thought to be responsible in part for pearl formation. Jameson (1902) believed that most mussel pearls resulted from encystment of metacercariae and encapsulation by the host. Herdman (1904), who studied sea mussels in England, found pearls to be very common near Piel and attributed them to invasion by larvae of *Distomum* (*Gymnophallus*) *somateriae*. Members of the family to which the parasite belongs—Gymnophallidae—are exclusively marine, utilizing bivalves as first and second intermediate hosts and birds as definitive hosts (Stunkard and Uzmann, 1959; James, 1964).

The literature on trematode-induced pearl formation in mussels has been reviewed by Stunkard and Uzmann (1958). The relationship between pearls in European sea mussels and trematode parasites was first described by Garner (1872) and later by Dubois (1901,b, 1903, 1907a,b, 1909), who proposed the name *Distomum* (*Gymnophallus*) *margaritarum* for parasites found in reddish—brown spots, which served as foci for pearl formation in mussels from the French coast. Jameson (1902) stated that the larval trematodes resembled *D. somateriae*, which has been described as an adult from the intestine of the eider duck, *Somateria mollissima*, by Levinsen (1881). Jameson (1902) described the process of pearl formation by the mantle of the mussel around the metacercariae. Giard (1903, 1907) confirmed these observations on pearl formation, and Odhner (1905) designated the larvae causing pearl formation in mussels as *Gymnophallus bursicola*. Similar metacercariae were found by Stafford (1912) in mussels from the Gulf of St. Lawrence. Jameson and Nicoll (1913) reviewed pearl formation in mussels and concluded that several gymnophallid larvae were involved. Since then, other gymnophallid cercariae have been associated with metacercariae in mussels (Palombi, 1924; Cole, 1935, Rees, 1939). Stunkard and Uzmann (1958) fed mussels from Long Island, New York, to newly hatched eider ducks and recovered adult gymnophallids, probably *G. bursicola*.

Other larval trematodes have been described from mussels. Cole (1935) reported "orange sickness," a fatal disease of sea mussels, at Conwy in Wales. The color was due to masses of orange-pigmented trematode sporocysts in the mantle and throughout the body; the tail-

less cercariae they contained were described as *Cercaria tenuans*. A similar condition had been noted previously by Atkins (1931). Uzmann (1953) found microcercous trematode larvae in sea mussels from Long Island and Connecticut that had a similar orange coloration. Described as *Cercaria milfordensis*, the larvae were primarily parasites of the blood vascular system of the host and had foci in the blood vessels of the mantle. Sporocyst development precluded normal gametogenesis in mussels; infected mussels held in aquaria had unusually high mortalities. Uzmann suggested that *C. milfordensis* infections were probably lethal to the host under unfavorable environmental conditions. Stunkard and Uzmann (1959) later identified progenetic stages of the worm in the mussel as *Proctoeces maculatus*. A similar trematode was reported by Hopkins (1954b) in the hooked mussel, *Brachidontes recurvus*, from Louisiana; cercariae developed in orange-pigmented sporocysts which completely destroyed the gonad of the mussel. Yamaguti (1938) identified as *Proctoeces* larval trematodes from the mussel *Brachidontes senhauisi* from Japan. Another species of *Proctoeces* was discovered in the Japanese pearl oyster, *Pinctada fucata*, in which parasitization caused significant damage (Sakaguchi *et al.*, 1970a,b; Sakaguchi, 1972). The definitive host proved to be the black porgy, *Mylio macrocephalus*.

Proctoeces maculatus, then, is a cosmopolitan species, capable of completing its life cycle and causing several pathology and mortality in bivalves such as the mussels *Mytilus edulis* and *Mytilus galloprovincialis* (Dupouy and Martinez, 1973; Martinez, 1973; Machkevsky and Parukhin, 1981) and encompassing larvae formerly described as *C. milfordensis*, *C. tenuans*, *C. brachidontis*, and *Proctoeces subtenuis* (Bray and Gibson, 1980).

Several life history stages of diverse trematodes occur in the soft-shell clam, *Mya arenaria*. Uzmann (1952) reported sporocysts and cercariae (*Cercaria myae*) in gonads and the digestive gland of this clam from Massachusetts, and held that parasitization resulted in a condition known as "water belly." [Subsequent observations, summarized by Dow and Wallace (1961), suggested that this condition may be a general sign of physiological disturbance.] Uzmann considered *C. myae* to be the same species as that reported by Stafford (1912) from the soft-shell clam in the Gulf of St. Lawrence. Hutton (1953) believed that the larvae were members of the genus *Gymnophallus*. Stunkard and Uzmann (1958) discussed gymnophallid sporocysts and cercariae in the soft-shell clam and attempted to unravel the taxonomy of the group.

The parasitological study of the soft-shell clams also included representatives of another trematode family—the Echinostomatidae. Stunkard (1960) found echinostome metacercariae of the genus *Himasthla* in

palps and gills of clams from the Maine coast. Three species were recognized. Earlier, Stunkard (1938) had demonstrated experimentally that cercariae of *Himasthla* would penetrate and encyst in the gills of *Mya arenaria* and a number of other bivalves, and Uzmann (1951) reported the natural occurrence of *Himasthla quissetensis*, with up to 100% prevalence in *M. arenaria*. Susceptibility and response of a number of marine pelecypods, including four species of clams, to cercariae of *H. quissetensis* were tested experimentally by Cheng *et al.* (1966). Metacercariae were found in all clams and mussels, but oysters were refractory to infection. *Himasthla* spp., as pointed out by Lauckner (1983), can invade clams in great numbers, they may kill young hosts, and they have been implicated in human gastrointestinal disturbances after ingestion of raw clams (Stunkard, 1938; Cheng, 1965). *Himasthla elongatus* metacercariae are important parasites of European cockles, to the extent that they have been proposed as controlling factors in population abundance in some areas (Lauckner, 1983).

Several larval trematodes have been reported in clams of the genus *Donax*. Giard (1897, 1907) identified bucephalid and gymnophallid cercariae. Rees (1939) found gymnophallid metacercariae, and Young (1953) described the life cycle of a monorchid, *Postmonorchis donacis*, whose larvae occur in the California bean clam, *Donax gouldi*. Hopkins (1958) identified sporocysts and cercariae of three species and metacercariae of two species in coquina clams, *Donax variabilis*, from the Texas coast. Infections by larval trematodes were considered by Pelseneer (1896, 1906, 1928) to be responsible for a reduced abundance of *Donax vittatus* in France, and Coe (1946) held that trematode parasites (*P. donacis*) were important in controlling population size in Californian *D. gouldi*. Subsequent studies disclosed high prevalences of *Postmonorchis* metacercariae in *D. gouldi*, causing sterilization of hosts and possibly being implicated in "population crashes," for which the clam is noted (Coe, 1953, 1955, 1956; Young, 1953; Johnson, 1968). Larval trematode parasites also cause sterilization of asari clams, *Tapes philippinarum*, in Japanese waters (Fujita, 1906, 1907, 1943).

Hopkins (1957a), in a brief but excellent exposition of the role of parasitism in marine communities, referred to an interesting interrelationship of host, parasite, and hyperparasite in the case of *Donax trunculus* parasitized by trematodes, which in turn were parasitized by the haplosporidan *Urosporidium pelseneeri*. The often severe fluctuations in the abundance of this clam have been attributed to shifts of balance in this tripartite relationship (Caullery and Chappellier, 1906; Cépède, 1911; Pelseneer, 1928). Other haplosporidan and microsporean hyperparasites of *Donax* have also been described (Guyénot *et al.*, 1925; Doll-

fus, 1946; Mackin and Loesch, 1955). One notable example is hyperparasitization of *Gymnophallus strigatus* metacercariae in *D. vittatus* from France by the microsporean *Nosema legeri* (Dollfus, 1912). Spores of the protozoan hyperparasite completely replace metacercarial tissues.

As is true in bivalves, larval trematode parasitization can be significant in gastropod species of economic importance, so the pervasive and, at times, severe effects of larval trematode infection on the abundance and reproduction of marine snails and snail populations must not be underestimated. Such parasitization almost invariably sterilizes the host, inhibits its seasonal migrations, and often leads to its early death (Cheng *et al.*, 1973; Williams and Ellis, 1976). Infections vary seasonally and geographically (Sindermann, 1960, 1965; Sindermann and Farrin, 1962; Lambert and Farley, 1968), but may at times involve a significant part of the snail population. Sindermann and Farrin found, for example, a general average of 52% of the periwinkle (*Littorina littorea*) population at a New England study site infected by *Cryptocotyle lingua*, whereas a coastwide sampling of the species in the Gulf of Maine disclosed an average midtide zone prevalence of 11%. Seasonal peaks of infection varied in different tide zones, partly because infected snails did not participate fully in normal spring and autumn migrations. Many infected individuals remained behind in the harsh winter high-tide zone environment of the northern New England coast.

Effects of larval trematode infections on survival of gastropods are variable. Some, such as *Renicola roscovita* in *L. littorea*, are considered lethal (Robson and Williams, 1971), whereas *C. lingua* seems less pathogenic to the same host species (Lauckner, 1983).

On the positive side of the ledger, larval trematodes have been looked to optimistically as a biological control method for harmful gastropods—especially for oyster drills (Cole, 1935; Carriker, 1955). Larval trematode parasitization was examined as a possible method of biological control of the southern oyster drill, *Thais haemostoma*, by Cooley (1958, 1962). Normal infection levels in natural populations of *Thais* were about 2% in Florida, 3.4% in Mississippi, and less than 1% in Texas. Infected individuals were sterilized by massive invasion of the gonads (Hopkins, 1957a). Cooley (1962), upon concluding the study, was not optimistic about use of the trematode *Parorchis acanthus* as a biological control measure because (1) the prevalence of adult worms in gulls (the definitive hosts) was low, (2) heavy infections of drills by experimental exposures were not obtained, and (3) drills not near gull feeding grounds were lightly infected, if at all. *Parorchis acanthus* has also been reported to cause sterilization of the most significant oyster drill of the Atlantic coastal states, *Urosalpinx cinerea* (Carriker, 1955), and has been

found in another muricid, *Purpura (Thais) lapillus*, in North America and in Europe (Stunkard and Shaw, 1931; Rees, 1937, 1939, 1940). Prevalence of the parasite reached 69% in some European populations, and altered sex ratios were observed (Feare, 1970).

Another aspect of larval trematode parasitization of marine gastropods—one with public health significance—is that some larval avian schistosomes can produce dermatitis in humans, as well as causing sterilization and inhibition of migration of the snail host. Known only since 1942 (Penner, 1942, 1950), avian schistosomes from marine snails have been described on both coasts of the United States and elsewhere (Hutton, 1952; Stunkard and Hinchliffe, 1952; Sindermann and Gibbs, 1953; Chu and Cutress, 1954; Grodhaus and Keh, 1958; Hutton, 1960; Sindermann, 1960; and others). One such trematode, *Austrobilharzia variglandis*, parasitizes the mud snail, *Nassarius obsoletus*, and a number of migratory birds on the United States East Coast. Infections are highest in the high-tide zone (Sindermann, 1960), with a peak in autumn, due in part to inhibition of normal offshore migration at that time of year. The highest prevalence observed was 27%. Greatest cercarial emergence, hence the greatest risk for human infection, occurred at intermediate salinities (15–25%). Emergence was inhibited at temperatures below 10°C. The same larval trematode has been reported from *Littorina planaxis* and from introduced *N. obsoletus* in California (Penner, 1950; Grodhaus and Keh, 1958), *Littorina pintado* in Hawaii (Chu and Cutress, 1954), and *Haminoea antillarum guadalupensis* in Florida (Hutton, 1952).

B. Cestodes

Oysters in several regions of the world are parasitized by larval cestodes usually, but possibly incorrectly, considered to be members of the genus *Tylocephalum*. Some authors consider *Tylocephalum* to be a junior synonym of *Tetragonocephalum* (Baer, 1948; Freeman, 1983), but since much of the literature refers to *Tylocephalum*, that designation will be used here. These parasites are Lecanicephalidea that occur as adults in the digestive tracts of elasmobranchs. Sparks (1963) reported heavy infections by *Tylocephalum* in American oysters introduced in Hawaii. In an addendum Sparks noted that oysters from Florida had been reported by the National Marine Fisheries Service Biological Laboratory, Oxford, Maryland, to harbor similar larval cestodes. Similar larvae have been found in oysters from Georgia and from North Carolina (Sindermann and Rosenfield, 1967). The coracidium of *Tylocephalum* was reported in the stomach and the gills of American oysters collected at Pearl Harbor, Hawaii (Cheng, 1966). Penetration of gill or digestive epithelium was

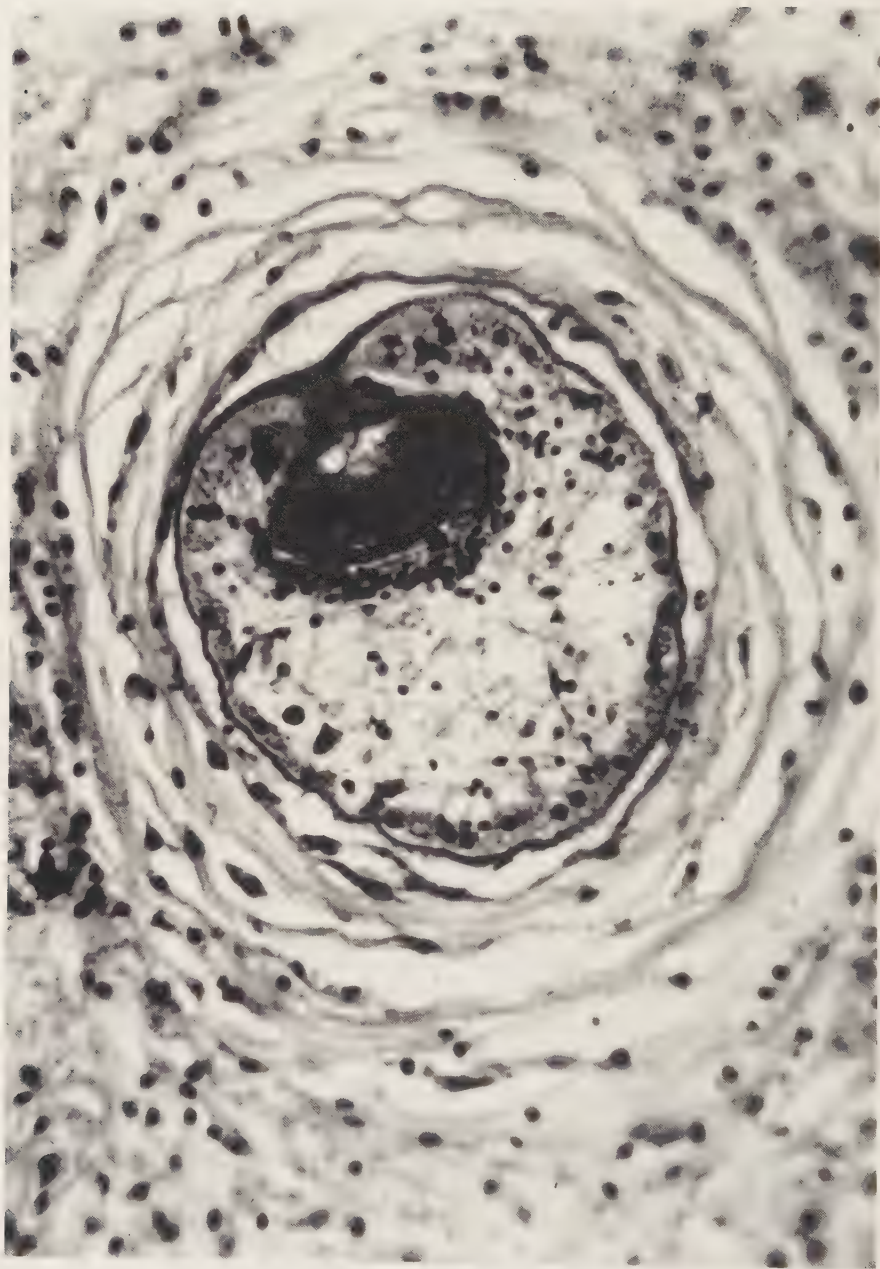


Figure 25. Larval tapeworm *Tylocephalum* in tissues of the American oyster.

postulated from the study of histological sections. The pronounced cellular reaction (Fig. 25) in the subepithelial tissues, including encapsulation of the larvae, was described (Rifkin and Cheng, 1968; Rifkin *et al.*, 1969). Larval cestodes, probably *Tylocephalum*, have also been found in Pacific oysters, *Crassostrea gigas*, from Japan and Taiwan by staff members of the National Marine Fisheries Service Biological Laboratory and from Hong Kong and China by Cheng (1975b). Two species of edible oysters from Australia were also reported to harbor *Tylocephalum* larvae, thereby extending an already broad geographic distribution (Wolf, 1976). Members of this tapeworm group are known as parasites of pearl oysters, *Margaritifera vulgaris*, in the Far East and were held to be the cause of pearl formation (Herdman, 1904; Herdman and Hornell, 1906; Shipley and Hornell, 1904, 1906; Southwell, 1924). Jameson (1912) presented convincing evidence, however, that invasion by larval trematodes, rather than larval cestodes, was more important to pearl development. Pearl formation around trematode and cestode larvae, which invade the mantle and die, appears to follow a similar pattern of host responses in bivalve molluscs (Wright, 1966). *Tylocephalum* larvae have also been reported from the Manila clam, *Tapes semidecussata*, from Hawaii. Extensive encapsulation, similar to that seen in edible oysters, leads to disintegration of the parasite (Cheng and Rifkin, 1968). Some generalizations about *Tylocephalum* larvae are that they are not host specific; they may occur in high prevalences and intensities, especially in pearl oysters and edible oysters; and they may reduce the condition of the molluscan host (Lauckner, 1983).

Other kinds of larval cestodes may parasitize bivalves, sometimes in overwhelming numbers. For example, Sparks and Chew (1966) described remarkable levels of parasitization of little neck clams, *Prototheca staminea* from California by larval tetraphyllidean cestodes of the genus *Echeneibothrium*. Cysts of the worm were closely packed throughout the tissues of the clams, which were abnormally exposed on the surface of gravel beds. Adult *Echeneibothrium*, with bothridia similar to those of larvae in clams, were found in bat stingrays, *Myliobatis californicus*, caught in the same area. A later study disclosed infections with *Echeneibothrium* sp. in the rough-sided little neck clam, *Prototheca laciniata*, also from California waters (Warner and Katkansky, 1969; Katkansky and Warner, 1969). Much earlier, larval cestodes (probably also *Echeneibothrium*) were found encysted, occasionally in large numbers, in the foot muscles of the gaper clam, *Tresus nuttalli* (MacGinitie and MacGinitie, 1949). The authors identified the bat stingray as the definitive host. A survey of gaper clams in one California estuary 20 years later

(Katkansky *et al.*, 1969) disclosed prevalences of encysted larvae approaching 100%.

Larval cestodes may be more abundant in molluscs than has been assumed. One study disclosed the presence of 11 species of larvae, representing four cestode orders, in molluscan hosts from the Gulf of Mexico (Cake, 1973, 1975, 1976, 1977a,b, 1979; Cake and Menzel, 1979, 1980). An important finding of the survey was that larval *Parachristianella* sp., a trypanorhynchan cestode found in penaeid shrimp, occurred frequently in bivalves (e.g., scallops and clams) as well. Encapsulated larvae were found in the walls of the digestive tract; heavy infections were thought to block food passage, partially or completely. Other than *Parachristianella* sp., a number of genera were identified—especially members of the order Tetraphyllidea, which, with the Trypanorhyncha, utilize elasmobranchs as definitive hosts.

Larval cestodes that mature in elasmobranch fish are also common parasites of cephalopods. Cestodes dominated the list of parasites reported by Clarke (1966), who summarized information on the parasites of oceanic squids, particularly the ommastrephids. MacGinitie and MacGinitie (1949) found jumbo squid, *Dosidicus gigas*, to be heavily infected with larval tetraphyllidean cestodes. Larvae occurred in the liver and the digestive tract. Other larval tapeworms of the genus *Tetrarhynchus* were found in tissues of the same squids. Larvae of the cestode family Tetrarhynchidae were also found by Adam (1938) in the mantle cavity of an octopus (*Octopus* sp.) from the Indian Ocean, and Dollfus (1923, 1927, 1929) reported members of the same group in a variety of cephalopods, including five species of squids and *Octopus vulgaris*. Dollfus (1958) published an extensive list of copepod, isopod, and helminth parasites of cephalopods from the Mediterranean Sea and the Atlantic Ocean. Larval cestodes, particularly trypanorhynchids, were most abundantly represented. Cestode larvae have also been found in cephalopods by Squires (1957) and by Clarke and Maul (1962).

C. Nematodes

Nematodes are neither conspicuous nor abundant parasites of marine molluscs—with a few exceptions, which have been studied intensively. These exceptions include members of the gnathostomid genus *Echinocephalus* from oysters, scallops, and abalones, and an ascaridoid, *Sulcascaaris sulcata*, from scallops and clams. All of the worms occur as larvae in molluscs, with adults in the predators of molluscs (Cheng, 1978).

Echinocephalus spp. occur in a number of marine molluscs. *Echinocephalus uncinatus*, for example, has been found in the adductor mus-

cle of the pearl oyster, *Margaritifera vulgaris*, and in other bivalves (Baylis and Lane, 1920). *Echinocephalus pseudouncinatus* was described in the California pink abalone, *Haliotis corrugata* (Millemann, 1951), and later from the scallop, *Argopecten aequisulcatus* from the same general area. *Echinocephalus sinensis* (= *Echinocephalus crassostreae*) was reported from oysters, *Crassostrea gigas*, being grown near Hong Kong (Ko *et al.*, 1975; Cheng, 1975a,b). Interest in the genus is derived in part from the possibility that at least some species may have public health significance as potential invaders of the human digestive tract.

Echinocephalus pseudouncinatus, found in California pink abalone, caused pronounced changes in the host. Heavy infections of older specimens were found; the worms encysted in the ventral part of the foot, producing external vesicles. The presence of blisters and the burrowing of the larvae were considered by Millemann (1951) to weaken the foot as a holdfast organ. The disease was known to commercial divers by gross appearance and the ease of removal of parasitized animals from rocks. The green abalone from the same area can also be infected, according to reports from processors and divers. The larval worms average 18 mm in length. Adult *E. pseudouncinatus* were found by Millemann (1963) in the horned shark, *Heterodontus francisci*, and the bat stingray, *Myliobatis californicus*, from the Gulf of California. Recovery of early juvenile stages of the nematode from teleost fishes (Shipley and Hornell, 1904; Johnston and Mawson, 1945) indicates that elasmobranchs may become infected by the ingestion of molluscan intermediate hosts or teleost transport hosts.

Echinocephalus sinensis, found in Pacific oysters from Hong Kong, localized in the visceral mass, close to the digestive diverticula, producing macroscopic green nodules containing coiled larvae (Ko *et al.*, 1975). The prevalence of infection in 1973—the year of the study—was 31%, with seasonal variations (a peak in August) and an increase in intensity of parasitization with advancing age of the host. A strong host cellular response characterized many infections, with massive hemocytic invasion of tissues surrounding the worm. Adult worms were found in 11 of 15 eagle rays, *Aetabatus flagellum*, sampled near the source of infected oysters (Ko, 1975). Attempts to infect mammals with larvae from oysters were successful. Worms penetrated to the muscularis externae of the digestive tracts of kittens and monkeys (Ko *et al.*, 1975). In a later study infections in mammals were achieved only during the warmer months of the year, and random visceral migration of larvae was seen (Ko, 1976).

Surf clams, *Spisula solidissima*, from the east coast of the United States are a principal economic resource, with an annual yield in excess of 30,000 tons. It was a matter of some concern, then, when reports of "brown worms" in surf clam meats began to proliferate in the mid-1970s. Hastily



Figure 26. Nematode *Sulcascaris sulcata* (hyperparasitized by *Urosporidium spisuli*) in an adductor muscle of the surf clam. (Courtesy of Mr. F. G. Kern.)

organized studies disclosed the presence of ascaridoid nematode larvae, *Sulcascaris sulcata*, in clam populations from New England to Cape Hatteras, with peak prevalences averaging 32% in Virginia waters and much lower levels further north (Kern, 1977). The larvae were usually white to orange and coiled in the muscle tissue. However, they were often hyperparasitized by a haplosporidan (ascetosporan) *Urosporidium spisuli*, and the presence of the protozoan spores turned the worm conspicuously dark brown (Fig. 26)—hence the problem (Perkins, 1971; Perkins *et al.*, 1975). The epizootic of the hyperparasite occurred in *S. sulcata* during 1974–1976, with infections in the Middle Atlantic states (especially Virginia and Maryland) sometimes approaching 100%, but with a sharp decline in 1976 (Payne *et al.*, 1980). Because of the unsightly condition of clam meats containing infected worms and because of some uncertainty about infectivity of the worms to humans, the clam industry was justifiably concerned, and clams were reported to be withheld from commercial trade in Maryland (Perkins *et al.*, 1975). Life cycle studies—part of a major cooperative research project—disclosed that the definitive hosts of the nematode were sea turtles (*Chelonia mydas* and *Caretta caretta*) (Sawyer *et al.*, 1983). The life cycle of the worm had been elucidated earlier, using

other molluscan intermediate hosts (Sprent, 1977; Berry and Cannon, 1981). These studies, indicating a reptilian rather than a mammalian or avian definitive host, when combined with the sharp reduction in abundance of the haplosporidan hyperparasite (and possibly reduction in the nematode population as well), resulted in lowered visibility of the problem, even though *Sulcascaris sulcata* continues as a significant parasite of *Spisula solidissima*.

The larval nematode parasitizes other molluscs as well. It occurs in the adductor muscles of the calico scallop, *Argopecten gibbus*, and the bay scallop, *Argopecten irradians*, from the east coast of the United States (Cobb, 1930; Hutton, 1964; Cheng, 1967; Lichtenfels *et al.*, 1976, 1978, 1980). Scallops, *Amusium balloti*, from Australia, are also infected with larval *S. sulcata*, with reported prevalences of up to 64%, sometimes resulting in reduced commercial value of the scallops (Sprent, 1977; Cannon, 1978; Lester *et al.*, 1980; Berry and Cannon, 1981). Gastropods may also be parasitized by the larval nematode. For example, the horse conch, *Pleuroploca gigantea*, and the banded tulip shell, *Fasciolaria lilium hunteria*, from Florida waters harbor the worms. Thus far, though, only larvae from surf clams have been found to be hyperparasitized by the haplosporidan *Urosporidium spisuli*.

Squids may also harbor larval nematodes. Squires (1957) and Clarke and Maul (1962) found larval anisakid worms in *Illex illecebrosus* and *Lepidoteuthis grimaldii*.

D. Summary

Although certainly not ranking in importance with the Protozoa in terms of effects on abundance of marine molluscs, the helminths—especially the larval trematodes—can reduce fecundity and debilitate hosts. The descriptive literature on larval trematodes is vast, but much of it concerns taxonomic and life history studies, with only a “dusting” of papers demonstrating serious pathology. Genera of significance in oysters include *Bucephalus*, *Gymnophalloides*, and *Proctoeces*; in mussels, *Gymnophallus* and *Proctoeces*; and in clams, *Himasthla*, *Gymnophallus*, and *Postmonorchis*.

Comparatively few larval cestodes or nematodes can be considered agents of disease in marine molluscs. Of the cestodes, those which mature in elasmobranchs are most abundant as larvae in molluscs—especially the genera *Tylocephalum* and *Echeneibothrium*. Nematode genera of significance are *Echinocephalus* and *Sulcascaris*, although other representatives have been reported.

A few members of other helminth groups may damage molluscs.



Figure 27. Extensive *Polydora* invasion of inner shell surfaces of *Crassostrea gigas*.

Prominent in this respect are polychaete annelids—of the genus *Polydora* in particular—that burrow into shells and are called mud worms or blister worms. Oysters, mussels, scallops, and other bivalves may be affected (Fig. 27), and mortalities may result from severe invasion (Lauckner, 1983). Also, large nemertean worms of the genus *Malacobdella* may occur in the mantle cavities of clams and other bivalves, but their pathogenic role is arguable.

Thus, although the literature on worm parasites of marine molluscs seems extensive, mostly because of long-term fascination of parasitologists with larval trematodes, the available information on cestodes and nematodes is very thin; and the combined data on helminths as disease agents indicate that the most serious problems occur in those molluscs which are first intermediate hosts of trematodes.

III. DISEASES OF CRUSTACEANS CAUSED BY HELMINTHS

Marine decapod crustaceans of economic importance are parasitized by an array of helminths, but few of them are seriously pathogenic except for isolated instances of heavy worm burdens in individual hosts. Interpret-

ing the term "helminth" broadly, crustaceans may be invaded by trematode metacercariae, larval trypanorhynchan cestodes, larval acanthocephalans, larval nematodes, an endoparasitic turbellarian, ectoparasitic nemerteans, and even leeches and other annelids—presenting an interesting spectrum of mostly immature worms, usually with only modest effects on their hosts. Unlike the situation in molluscs as first intermediate hosts of trematodes, with frequent extensive destruction of gonads, only weak counterparts of effects on reproduction occur in crustaceans: in the destruction of crab eggs by parasitic nemerteans or sterilization of shrimp by an endoparasitic turbellarian. Evidence for a direct association of mortality of crustaceans with helminth parasitization is sparse, even though some reduction in host vitality is a common accompaniment of parasitization.

This section on helminths of crustaceans is brief, a reflection of their relative significance as agents of severe disease.

A. Trematodes

Trematode metacercariae are common in several species of crabs. Larvae of *Microphallus nicolli* and *M. basodactylophallus* infect body muscles, hepatopancreas, and ventral ganglia of blue crabs *Callinectes sapidus* and *Callinectes similis* (Cable and Hunninen, 1940; Overstreet, 1983). Adult *M. nicolli* occur in young herring gulls, *Larus argentatus*, and sporocyst generations in the snail *Bittium alternatum*. A haplosporidan (ascetosporean) hyperparasite, *Urosporidium crescens*, has been found in metacercariae from blue crabs (De Turk, 1940; Couch, 1974; Overstreet, 1978). One third of the crabs sampled in the earliest study contained metacercariae, but the extent of hyperparasitization was not determined. The host metacercarial tissue was often completely destroyed, leaving little more than a sack of protozoan spores. Invasion by *Urosporidium* was thought to occur before encystment of cercariae. Masses of dark-pigmented spores in the destroyed metacercariae produce conspicuous black spots, which has led to the application of the descriptive colloquial term "pepper crab" to hyperparasitized individuals. Pepper spots occur most commonly in fat bodies, the digestive gland, and muscles of the crab.

Metacercariae of *Microphallus carcini* have been observed in the hepatopancreas of green crabs from the English Channel (Guyénot *et al.*, 1925) and from the Mediterranean Sea (Timon-David, 1949). Hyperparasitization of metacercariae by the microsporean, *Nosema (Pleistophora) spelotremae*, was reported by Guyénot *et al.* (1925). Metacercariae of another microphallid, *Microphallus similis*, parasitize green crabs of the

western North Atlantic Ocean (Stunkard, 1957). Young green crabs were killed in 10–20 days by massive experimental exposures to cercariae of *M. similis*.

Trematode metacercariae have also been seen encysted within the nerves of Dungeness crabs, *Cancer magister*, from the northwestern United States (Sparks and Hibbits, 1981). A pronounced host response occurred if ganglia were invaded, and necrosis was a consistent finding. Identity of the worm was not determined, but resemblances to members of the genus *Microphallus* were noted (Sparks, 1985).

Shrimp occasionally harbor larval trematodes. Metacercariae of *Opecoeloides fimbriatus* were reported from numerous organs and were abundant in penaeids from the southeastern United States (Hutton *et al.*, 1959; Sogandares-Bernal and Hutton, 1959; Overstreet, 1973). Another larval trematode, *Microphallus* sp., has been reported from body muscles and the hepatopancreas of pink shrimp from Florida (Hutton *et al.*, 1959).

Immature aspidogastrid trematodes, *Stichocotyle nephropis*, encyst in the stomach and the intestinal walls of lobsters, *Nephrops norvegicus* and *Homarus americanus*, from Europe and from North America (Cunningham, 1887; Nickerson, 1894; Herrick, 1895; Odhner, 1910; Montreuil, 1954; MacKenzie, 1963). Montreuil found the parasite in lobsters taken near the mouth of the Bay of Fundy in the Gulf of Maine but not in more than 500 lobsters examined from the Gulf of St. Lawrence. Adult *S. nephropis* parasitize several species of skates and rays (Odhner, 1898; Linton, 1940).

B. Cestodes

Several larval cestodes of the order Trypanorhyncha have been found in the digestive gland and other organs of shrimp. Larval *Prochristianella penaei* have been identified from four species of shrimp (brown, *Penaeus aztecus*; white, *Penaeus setiferus*; pink, *Penaeus duorarum*; and humpback, *Trachypenaeus constrictus*) from the Gulf of Mexico (Sparks and Mackin, 1957; Woodburn *et al.*, 1957; Hutton *et al.*, 1959; Kruse, 1959; Aldrich, 1965). The adult worm was identified from the Atlantic stingray, *Dasyatis sabina*. The prevalence in shrimp from the western Gulf averaged 48% in one study (Aldrich, 1965), with some samples exceeding 90% and with pronounced geographic variations. Their condition seemed unaffected, even for heavily parasitized animals (Ragan and Aldrich, 1972). Other species of larval trypanorhynchan cestodes have been reported from Gulf of Mexico shrimp; *Parachristianella monomegacantha* and *Parachristianella dimegacantha* were both described in pink shrimp and later in brown shrimp (Kruse, 1959; Corkern, 1970).

Other unidentified larval cestodes have been seen, sometimes in great numbers (>1000) in the gut, abdominal muscles, gills, and pericardium of shrimp from the Gulf of Mexico (Ward, 1962; Villella *et al.*, 1970; Overstreet, 1973). Some of these may have been lecanocephalids, possibly *Polypocephalus* sp., but their definitive host is unknown.

Trypanorhynch larvae have been found in commercial shrimp from other parts of the world. Yamaguti (1934) reported larvae, probably *Tettrarhynchus rubromaculatus*, in *Penaeopsis* sp. from Japan. Heldt (1949) took a larval cestode resembling *Eutetrarhynchus ruficollis* from *Penaeus trisulcatus* from the North African coast.

C. Parasitic Turbellarians

A large endoparasitic turbellarian, *Kronborgia caridicola*, has been found in North Atlantic shrimp from Greenland by Kanneworff and Christensen (1966). The parasitic female lies tightly coiled and occupies much of the body cavity of the host, rendering the shrimp sterile and probably killing it before the mature worm leaves to secrete a cocoon and deposit egg capsules. Many cocoons were collected from level shrimp bottoms. Infected shrimp of three species, *Lebbeus polaris*, *Eualus machilenta*, and *Paciphaea tarda*, were taken by commercial shrimp trawlers.

D. Acanthocephalans

A larval acanthocephalan, probably of the genus *Corynosoma*, was identified in American lobsters from the Gulf of St. Lawrence and elsewhere in the Canadian Maritime Provinces by Montreuil (1954). The worms were usually encysted in the thin wall of the intestine, although some had apparently perforated the gut and encysted in the heart and body muscles. Montreuil believed that accidental gut perforation may provide a route of entry for secondary invaders and could account for appreciable mortality. Feeding experiments with cats and seals suggested that the stage of *Corynosoma* in the lobster is not infective to mammals. In a later study lobsters from inshore waters of the Gulf of Maine were also found to be infected by juvenile *Corynosoma* sp. (Uzmann, 1970).

Crabs may also harbor larval acanthocephalans. Rock crabs, *Cancer irroratus*, from the Maine coast were infected by *Polymorphus major*. The larvae were encysted in clusters attached to the midgut, and the definitive hosts were identified as diving ducks (Schmidt and MacLean, 1978).

E. Nematodes

Nematodes occur as commensals in egg masses of crabs, or as internal parasites. *Rhabdochona uca* parasitizes the digestive tract of *Uca mani*, and

Carcinus and *Pagurus* are intermediate hosts of nematodes that mature in elasmobranch fish (Gordon, 1966).

A larval nematode, tentatively assigned to the genus *Ascarophis*, has been reported from lobsters taken off the northeastern coast of the United States (Anonymous, 1966; Uzman, 1967a). Adults of the genus occur in fish, particularly gadoids (Uspenskaya, 1953). Larvae occurred commonly in lobsters from Georges Bank and several canyons along the edge of the continental shelf south of Cape Cod, Massachusetts, but were absent in lobsters from near the coast. The larvae were encysted in the rectal wall of 25% of the offshore lobsters examined by Uzman, who speculated that larvae from lobsters reach maturity in abundant cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) populations of Georges Bank. A later study disclosed comparable prevalences of *Ascarophis* in offshore lobsters from the Middle Atlantic Bight (Van Engel *et al.*, 1986).

Larval nematodes of the genus *Hysterothylacium* (formerly *Contracaecum*), which occur as adults in fish, were found in Florida shrimp by Woodburn *et al.* (1957), Hutton *et al.* (1959), and Kruse (1959). Margolis and Butler (1954) observed adult *Hysterothylacium* in a single specimen of northern pink shrimp, *Pandalus borealis*, from British Columbia, Canada—probably an example of an abnormal definitive host.

F. Nemerteans

Egg masses of crabs and lobsters may be destroyed by ectoparasitic nemertean worms (phylum Rhynchocoela). Juvenile nemerteans, *Carcinonemertes carcinophila*, encyst on the gills of the blue crab, *Callinectes sapidus* (Overstreet, 1978, 1983). After the female crab spawns, the worms excyst and migrate to the egg mass, where they feed on the host's eggs, mature, lay eggs, then return to the gills. The worm can serve as an indicator of host physiology; reencysted worms are larger and orange after the host has spawned (Hopkins, 1947; Hargis, 1959; Davis, 1965).

Another species, *Carcinonemertes errans*, occurs on Dungeness crabs, *Cancer magister*, and is known as a serious predator on crab eggs (Fig. 28). In one study of an apparent population explosion of the worm on the central California coast, estimated host egg mortality was 55% over a 5-year period (Wickham, 1979, 1980, 1986). Combined field and laboratory observations supported the estimates and demonstrated the severity of the problem. Egg masses of individual crabs could be infested by as many as 100,000 worms; heavily infested crabs lost all of their eggs, and the overall population of crabs lost over half of their eggs in most years.

The destructive role of nemerteans as egg predators of lobsters, *Homarus americanus*, has been reported (Aiken *et al.*, 1983, 1985; Bratney

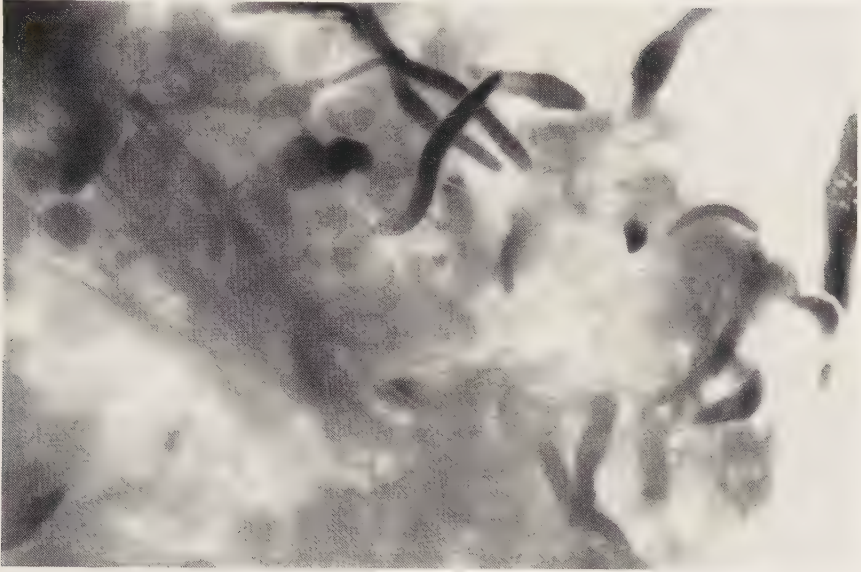


Figure 28. Nemertean worms, *Carcinonemertes errans*, on an egg mass of a Dungeness crab, *Cancer magister*. (Courtesy of Dr. D. E. Wickham.)

et al., 1985; Uhazy *et al.*, 1985). Egg masses of lobsters from the western North Atlantic Ocean were often found to be completely destroyed by feeding activities of *Pseudocarcinonemertes homari*, and severe effects on host populations were considered possible, but were not demonstrated (Campbell and Bratney, 1986).

Epizootic infestations of other crustaceans by nemertean brood parasites have been reported. Outbreaks have occurred in Pacific shore crabs, *Hemigrapsus oregonensis* (Shields and Kuris, 1983); in Alaskan red king crabs, *Paralithodes camtschatica*; and in Pacific tanner crabs, *Chionoecetes bairdi* (Wickham *et al.*, 1985; Wickham, 1986). Populations of worms varied in abundance drastically. In king crabs, for example, densities of the parasites were high enough in some areas and seasons to cause mortality of nearly all of the eggs brooded by the host crabs, whereas in other areas nemerteans were absent.

G. Leeches and Other Annelids

A localized mortality of blue crabs, thought to be caused by parasitization by leeches, *Myzobdella lugubris*, was reported in a Florida river by Hutton and Sogandares-Bernal (1959). A sample of seven crabs had 32 leeches attached near the base of the legs and near perforations in the

exoskeleton. The parasite is known from the Atlantic and Gulf coasts of the United States, but had not previously been considered to cause mortalities (Moore, 1946).

Other leeches occur on crabs. Oka (1927) described *Carcinobdella kanibir* on Japanese edible crabs, *Chionoecetes opilio*. Egg cases and adults of the leech *Notostomobdella cyclostoma* are common on Alaskan king crabs, particularly during the summer (Moore and Meyer, 1951; Bright *et al.*, 1960). Undescribed worms, probably leeches, were seen by MacKay (1942) on the abdomens of female Dungeness crabs, *C. magister*, from British Columbia. The worms were much larger on egg-bearing crabs and were found chiefly among the eggs.

Havinga (1921), in a discussion of artificial lobster rearing, described the attachment of a small green annelid worm, *Histriobdella homari*, to the eggs and to all parts of the bodies of larval and adult lobsters in Norway. He attributed poor success in the production of larvae to the effects of the worm. The same parasite had been observed earlier (Sund, 1914, 1915) in massive numbers on the eggs of lobsters held in floating boxes at Korshavn, Norway, where it was held responsible for destruction of the brood. Every female lobster was infested with thousands of worms, and they also occurred on lobster larvae. Although *H. homari* had not been reported previously from American lobsters, Uzmann (1967b) found it to be widely distributed on the gills of lobsters from New England coastal waters (Maine to Connecticut) and from Georges Bank.

H. Summary

Available information about helminth parasites of marine crustaceans of economic importance suggests relatively minor effects as disease agents. Once this generalization has been stated, a number of lesser but interesting observations can be made:

1. Larval trypanorhynchan cestodes (which mature in elasmobranchs) are probably the most abundant helminth parasites of marine shrimp. They may occur in high prevalences, and the intensity of infection may also be high. However, host reaction to the presence of larval cestodes is usually pronounced, with progressive encapsulation, followed by destruction and resorption of the worms.
2. Larval trematodes, usually of the family Microphallidae, occur commonly in crabs in Europe and in the United States. The hepatopancreas is a favored site for the encystment of microphallid larvae, although they may occur in other organs and tissues.

3. Hyperparasitization of larval helminths in Crustacea parallels that seen in certain helminths in molluscs. Microsporean and ascetosporan Protozoa may infect and destroy trematode parasites of crabs.

It seems, then, that the helminths are interesting but not especially pathogenic for marine crustaceans of economic importance, but that Crustacea serve as important intermediate hosts for trematodes, cestodes, and acanthocephala that mature in fish and in warm-blooded animals.

IV. CONCLUSIONS

This chapter on molluscan and crustacean shellfish diseases caused by helminths is clearly biased in favor of larval trematodes that infect molluscs, since these are the parasites of greatest consequence, as measured by the criterion of the effect on the host. The early life history stages of trematodes such as *Bucephalus*, *Proctoeces*, *Himasthla*, and *Postmonorchis* sterilize molluscan hosts and contribute to mortality, directly or indirectly. Marine molluscs also may act as second intermediate hosts for trematodes which mature in birds and in mammals; effects of metacercariae on the molluscan host are usually less severe, although massive infections by species such as *Himasthla elongatus* may affect survival. Crustaceans may also act as second intermediate hosts of trematodes which mature in fish or in warm-blooded animals, but the effects of metacercariae on the host seem relatively minor.

Larval cestodes which mature in elasmobranchs are common in molluscs, usually encysted in host tissues, where they elicit an intense cellular response. Genera which parasitize commercial bivalves, and hence have received attention, include *Tylocephalum*, *Echeneibothrium*, and *Parachristianella*. Larval trypanorhynchan cestodes also occur in shrimp, but seem to do little harm, even in heavy infections.

Larval nematodes occur sporadically in molluscs and crustaceans, but, like the cestodes, are not seriously pathogenic. Two genera—*Echinocephalus* and *Sulcascaris*—which parasitize a diversity of molluscs, have received scientific attention because of the economic importance of their hosts. Similarly, *Ascarophis* and *Hysterothylacium*, which parasitize lobsters and shrimp, have been studied.

Most of the helminths, then (except larval trematodes, which can be very destructive in molluscs), fail as significant pathogens of shellfish—with a role that must be rated only slightly better than trivial.

REFERENCES

- Adam, W. (1938). Sur la présence d'une larve de cestode (Tetrarhynchidae) dan la cavité palléale d'un *Octopus* des Iles Andamans. *Bull. Mus. R. Hist. Nat. Belg. (Brussels)* **14**, 1–4.
- Aiken, D. E., Waddy, S. L., Uhazy, L. S., and Campbell, A. (1983). A nemertean destructive to the eggs of the lobster, *Homarus americanus*. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **182**, 130–133.
- Aiken, D. E., Waddy, S. L., and Uhazy, L. S. (1985). Aspects of the biology of *Pseudocarcinonemertes homari* and its association with the American lobster, *Homarus americanus*. *Can. J. Fish. Aquat. Sci.* **42**, 351–356.
- Aldrich, D. V. (1965). Observations on the ecology and life cycle of *Prochristianella penaei* Kruse (Cestoda: Trypanorhyncha). *J. Parasitol.* **51**, 370–376.
- Anonymous (Uzmann, J. R.) (1966). Boothbay studies parasite of ocean lobster. *U.S. Fish Wildl. Serv., Fish Wildl. Rep.*, p. 21.
- Atkins, D. (1931). On abnormal conditions of the gills in *Mytilus edulis*. II. Structural abnormalities, with a note on the method of division of the mantle cavity in normal individuals. *J. Mar. Biol. Assoc. U.K.* **17**, 489–543.
- Baer, J. G. (1948). Contributions á l'étude des cestodes de sélaciens I–IV. *Bull. Soc. Neu-chatel. Sci. Nat.* **71**, 63–122.
- Baylis, H. A., and Lane, C. (1920). A revision of the nematode family Gnathostomatidae. *Proc. Zool. Soc. London* 245–310.
- Berry, G. N., and Cannon, L. R. G. (1981). The life history of *Sulcascaris sulcata* (Nematoda: Ascaridoidea), a parasite of marine molluscs and turtles. *Int. J. Parasitol.* **11**, 43–54.
- Bratney, J., Campbell, A., Bagnall, A. E., and Uhazy, L. S. (1985). Geographic distribution and seasonal occurrence of the nemertean *Pseudocarcinonemertes homari* on the American lobster, *Homarus americanus*. *Can. J. Fish. Aquat. Sci.* **42**, 360–367.
- Bray, R. A., and Gibson, D. I. (1980). The Fellodistomidae (Digenea) of fishes from the Northeast Atlantic. *Bull. Br. Mus. (Nat. Hist.), Zool.* **37**, 199–293.
- Bright, D. B., Durham, F. E., and Knudsen, J. W. (1960). King crab investigations of Cook Inlet, Alaska. *U.S. Bur. Comm. Fish. Biol. Lab., Auke Bay, Alaska, Contract Rep.* pp. 149–154 (unpublished).
- Cable, R. M., and Hunninen, A. V. (1940). Studies on the life-history of *Spelotrema nicolli* (Trematoda: Microphallidae) with the description of a new microphallid cercaria. *Biol. Bull. (Woods, Hole, Mass.)* **78**, 136–157.
- Cake, E. W. (1973). Larval cestode infections in several edible bivalve mollusks from the vicinity of St. Teresa, Florida. *Proc. Natl. Shellfish. Assoc.* **63**, 1.
- Cake, E. W. (1975). "Larval and Postlarval Cestode Parasites of Shallow-Water, Benthic Mollusks of the Gulf of Mexico from the Florida Keys to the Mississippi Sound," dissertation. Florida State University, Tallahassee, Florida.
- Cake, E. W. (1976). A key to larval cestodes of shallow-water, benthic mollusks of the northern Gulf of Mexico. *Proc. Helminthol. Soc. Wash.* **43**, 160–171.
- Cake, E. W. (1977a). Larval cestode parasites of edible mollusks of the northeastern Gulf of Mexico. *Gulf Res. Rep.* **6**, 1–8.
- Cake, E. W. (1977b). Experimental infection studies with bothridio-plerocercoids of *Rhinebothrium* sp. (Cestoda, Tetraphyllidea) and two intermediate molluscan hosts. *Northeast Gulf Sci.* **1**, 55–59.
- Cake, E. W. (1979). *Polypocephalus* sp. (Cestoda; Lecanicephalidae): A description of tentaculoplerocercoids from bay scallops of the northeastern Gulf of Mexico. *Proc. Helminthol. Soc. Wash.* **46**, 165–170.

- Cake, E. W., and Menzel, R. W. (1979). Infections of *Tylocephalum* in commercial oysters and three predaceous gastropods of the eastern Gulf of Mexico. *Proc. Natl. Shellfish. Assoc.* **69**, 193.
- Cake, E. W., and Menzel, R. W. (1980). Infections of *Tylocephalum* metacestodes in commercial oysters and three predaceous gastropods of the eastern Gulf of Mexico. *Proc. Natl. Shellfish. Assoc.* **70**, 94–104.
- Campbell, A., and Bratley, J. (1986). Egg loss from the American lobster, *Homarus americanus*, in relation to nemertean, *Pseudocarcinonemertes homari*, infestation. *Can. J. Fish. Aquat. Sci.* **43**, 772–780.
- Cannon, L. R. G. (1978). A larval ascaridoid nematode from Queensland scallops. *Int. J. Parasitol.* **8**, 75–80.
- Carriker, M. R. (1955). Critical review of biology and control of oyster drills *Urosalpinx* and *Eupleura*. *U.S. Fish Wild. Serv., Spec. Sci. Rep.—Fish.* **148**, 1–150.
- Caullery, M., and Chappellier, A. (1906). *Anurosporidium pelseneri*, n. g. n. sp., haplosporidie infectant les sporocysts d'un trématode parasite de *Donax trunculus* L. *C. R. Soc. Biol.* **60**, 325–328.
- Cépède, C. (1911). Le cycle évolutif et les affinités systématiques de l'haplosporidie des *Donax*. *C. R. Acad. Sci.* **153**, 507–509.
- Cheng, T. C. (1964). Studies on phosphatase systems in hepatopancreatic cells of the molluscan host of *Echinoparyphium* sp. and in the rediae and cercariae of this trematode. *Parasitology* **54**, 73–79.
- Cheng, T. C. (1965). Histochemical observations on changes in the lipid composition of the American oyster, *Crassostrea virginica* (Gmelin), parasitized by the trematode *Bucephalus* sp. *J. Invertebr. Pathol.* **7**, 398–407.
- Cheng, T. C. (1966). The coracidium of the cestode *Tylocephalum* and the migration and fate of this parasite in the American oyster, *Crassostrea virginica*. *Trans. Am. Microsc. Soc.* **85**, 246–255.
- Cheng, T. C. (1967). Marine molluscs as hosts for symbioses: With a review of known parasites of commercially important species. *Adv. Mar. Biol.* **5**, 1–424.
- Cheng, T. C. (1975a). *Echinocephalus crassostrei* sp. nov., a larval nematode from the oyster *Crassostrea gigas* in the Orient. *J. Invertebr. Pathol.* **26**, 81–90.
- Cheng, T. C. (1975b). New geographic records for *Tylocephalum* metacestodes. *J. Invertebr. Pathol.* **26**, 395–396.
- Cheng, T. C. (1978). Larval nematodes parasitic in shellfish. *Mar. Fish. Rev.* **40**(10), 39–42.
- Cheng, T. C., and Burton, R. W. (1965). Relationships between *Bucephalus* sp. and *Crassostrea virginica*: Histopathology and sites of infection. *Chesapeake Sci.* **6**, 3–16.
- Cheng, T. C., and Rifkin, E. (1968). The occurrence and resorption of *Tylocephalum* metacestodes in the clam *Tapes semidecussata*. *J. Invertebr. Pathol.* **10**, 65–69.
- Cheng, T. C., Shuster, C. N., Jr., and Anderson, A. H. (1966). A comparative study of the susceptibility and response of eight species of marine pelecypods to the trematode *Himasthla quissetensis*. *Trans. Am. Microsc. Soc.* **85**, 284–295.
- Cheng, T. C., Sullivan, J. T., and Harris, K. R. (1973). Parasitic castration of the marine prosobranch gastropod *Nassarius obsoletus* by sporocysts of *Zoogonus rubellus* (Trematoda): Histopathology. *J. Invertebr. Pathol.* **21**, 183–190.
- Ching, H. L. (1972). A redescription of *Gymnophalloides tokiensis* Fujita, 1925 (Trematode: Gymnophallidae). *Can. J. Zool.* **50**, 1299–1302.
- Chu, G. W. T. C., and Cutress, C. E. (1954). *Austrotilharzia variglandis* (Miller and Northup, 1926) Penner, 1953, (Trematoda: Schistosomatidae) in Hawaii with notes on its biology. *J. Parasitol.* **40**, 515–523.

- Clarke, M. R. (1966). A review of the systematics and ecology of the oceanic squids. *Adv. Mar. Biol.* **4**, 91–300.
- Clarke, M. R., and Maul, G. E. (1962). A description of the “scaled” squid *Lepidoteuthis grimaldi* Joubin 1895. *Proc. Zool. Soc. London* **139**, 97–118.
- Cobb, N. A. (1930). A nemic parasite of *Pecten*. *J. Parasitol.* **17**, 104–105.
- Coe, W. R. (1946). A resurgent population of the California bay-mussel (*Mytilus edulis* *diagensis*). *J. Morphol.* **78**, 85–103.
- Coe, W. R. (1953). Resurgent populations of littoral marine invertebrates and their dependence on ocean currents and tidal currents. *Ecology* **34**, 225–229.
- Coe, W. R. (1955). Ecology of the bean clam *Donax gouldi* on the coast of southern California. *Ecology* **36**, 512–514.
- Coe, W. R. (1956). Fluctuations in populations of littoral marine invertebrates. *J. Mar. Res.* **15**, 212–232.
- Cole, H. A. (1935). On some larval trematode parasites of the mussel (*Mytilus edulis*) and the cockle (*Cardium edule*). *Parasitology* **27**, 276–280.
- Cooley, N. R. (1958). Incidence and life history of *Parorchis acanthus*, a digenetic trematode, in the southern oyster drill, *Thais haemastoma*. *Proc. Natl. Shellfish. Assoc.* **48**, 174–188.
- Cooley, N. R. (1962). Studies on *Parorchis acanthus* (Trematoda: Digenea) as a biological control for the southern oyster drill, *Thais haemastoma*. *U.S. Fish Wildl. Serv., Fish. Bull.* **62**, 77–91.
- Corkern, C. C., Jr. (1970). “Investigations on Helminths from the Hepatopancreas of the Brown Shrimp, *Penaeus aztecus* Ives, from Galveston Bay, Texas,” M.A. thesis, 51 pp. Texas A&M University, College Station, Texas.
- Couch, J. A. (1974). Pathological effects of *Urosporidium* (Haplosporidia) infection in microphallid metacercariae. *J. Invertebr. Pathol.* **23**, 389–396.
- Cunningham, J. T. (1887). On *Stichocotyle nephropis*, a new trematode. *Trans. R. Soc. Edinburgh* **32**, 273–280.
- Davis, C. C. (1965). A study of the hatching process in aquatic invertebrates. XX. The blue crab, *Callinectes sapidus*, Rathbun. XXI. The nemertean *Carcinonemertes carcinophila* (Kölliker). *Chesapeake Sci.* **6**, 201–208.
- Deltreil, J.-P., and His, É. (1970). Sur la présence d’une cercaire de trématode chez *Cardium edule* L. dans le bassin d’Arcachon. *Rev. Trav. Inst. Pêches Marit.* **34**, 225–232.
- De Turk, W. E. (1940). The occurrence and development of a hyperparasite *Urosporidium crescens* sp. nov. (Sporozoa, Haplosporidia), which infests the metacercariae of *Spelotrema nicolli*, parasitic in *Callinectes sapidus*. *J. Elisha Mitchell Sci. Soc.* **56**, 231–232.
- Dollfus, R. P. (1912). Contribution à l’étude des trématodes marins des cotes du Boulonnais. Une métacercaire margaritifé parasite de *Donax vittatus* da Costa. *Mem. Soc. Zool. Fr.* **25**, 85–144.
- Dollfus, R. P. (1923). Énumération des cestodes du plancton et des invertébrés marins. II. Mollusques céphalopodes et crustacés. *Ann. Parasitol. Hum. Comp.* **1**, 363–394.
- Dollfus, R. P. (1927). Sur une métacercaire progénétique d’Hémiuridae (Trematoda: Digenea). *Bull. Biol. Fr. Belg.* **61**, 49–58.
- Dollfus, R. P. (1929). Addendum à mon “Énumération des cestodes du plancton et des invertébrés marins.” *Ann. Parasitol. Hum. Comp.* **7**, 325–347.
- Dollfus, R. P. (1946). Parasites des helminths. *Encycl. Biol.* **27**, 1–482.
- Dollfus, R. P. (1958). Copépodes, isopodes et helminthes parasites de céphalopodes de la Méditerranée et de l’Atlantique Européen. *Faune Mar. Pyrenées—Orientales* **1**, 61–72.
- Dow, R. L., and Wallace, D. E. (1961). The soft-shell clam industry of Maine. *U.S. Fish Wildl. Serv., Circ.* **110**, 1–36.

- Dubois, R. (1901a). Sur la mécanique de la formation des perles fines dans le *Mytilus edulis*. C. R. Acad. Sci. **133**, 603–605.
- Dubois, R. (1901b). Sur le mode de formation des perles dans *Mytilus edulis* L. C. R. Assoc. Fr. Adv. Sci. **1**, 149–150.
- Dubois, R. (1903). L'origine des perles chez le *Mytilus gallo-provincialis*. C. R. Acad. Sci. **136**, 178–179.
- Dubois, R. (1907a). Sur un sporozoaire parasite de l'huître perlière, *Margaritifera vulgaris* Jam., son rôle dans la formation des perles fines. C. R. Soc. Biol. **62**, 310–311.
- Dubois, R. (1907b). Sur les métamorphoses du distome parasite des *Mytilus* perliers. C. R. Soc. Biol. **63**, 335–336.
- Dubois, R. (1909). Contribution à l'étude des perles fines de la nacre et des animaux qui les produisent. Ann. Univ. Lyon, New Ser. **29**, 1–126.
- Dupouy, J., and Martinez, J.-C. (1973). Action de *Proctoeces maculatus* (Looss, 1901) (Trematode, Fellodistomatidae) sur le développement des gonades chez *Mytilus galloprovincialis* Lmk. C. R. Hebd. Séances Acad. Sci., Ser. D **227**, 1889–1892.
- Feare, C. J. (1970). Aspects of the ecology of an exposed shore population of dogwhelks *Nucella lapillus* (L.). Oecologia **5**, 1–18.
- Freeman, R. S. (1983). Pathology of the invertebrate host–metacestode relationship. In "Biology of the Eucestoda," Vol. 2, pp. 441–497. (C. Arme, and P. W. Pappas, eds.), Academic Press, London.
- Fujita, T. (1906). Two species of cercariae from *Paphia* (*Amygdala*) *philippinarum*. Dobutsugaku Zasshi **18**, 197–202 (in Japanese).
- Fujita, T. (1907). The specific name of the cercaria found in *Paphia* (*Amygdala*) *philippinarum*. Dobutsugaku Zasshi **19**, 281–282 (in Japanese).
- Fujita, T. (1925). Études sur les parasites de l'huître comestible du Japon *Ostrea gigas* Thunberg. Traduction accompagnée de notes, de diagnoses et d'une bibliographie, par Robert Ph. Dollfus. Ann. Parasitol. Hum. Comp. **3**, 37–59.
- Fujita, T. (1943). "Diseases of Fish and Shellfish." Koa Nippon Book Co., Tokyo (in Japanese).
- Garner, R. (1872). On the formation of British pearls and their possible improvement. J. Linn. Soc. London **11**, 426–428.
- Giard, A. (1897). Sur un cercaire sétigère (*Cercaria lutea*) parasite des pélicypodes. C. R. Soc. Biol. **49**, 954–956.
- Giard, A. (1903). Sur la production volontaire des perles fines, ou margarose artificielle. C. R. Soc. Biol. **55**, 1225–1226.
- Giard, A. (1907). Sur les trématodes margaritigènes du Pas-de-Calais (*Gymnophallus somateriae* Levinsen et *G. bursicola* Odhner). C. R. Soc. Biol. **63**, 416–420.
- Gordon, I. (1966). Parasites and diseases of Crustacea. Mem. Inst. Fond. Afr. Noire **77**, 27–86.
- Grodhaus, G., and Keh, B. (1958). The marine dermatitis-producing cercaria of *Austrobilharzia variglandis* in California (Trematoda: Schistosomatidae). J. Parasitol. **44**, 633–638.
- Guyénot, E., Naville, A., and Ponse, K. (1925). Deux microsporidies parasites de trématodes. Rev. Suisse Zool. **31**, 399–421.
- Hargis, W. J., Jr. (1959). Parasites and fishery problems. Proc. Annu. Sess. Gulf Caribb. Fish. Inst. **11th** 70–75.
- Havinga, B. (1921). Rapport over de kreeftenvisscherij in Zeeland en de Kunstmatige kreeftenteelt. Meded. Versl. Visscherijinspect. **30**, 51 pp.
- Heldt, J. H. (1949). Notes sur la présence d'une pléroceroïde de tetrarhynque dans

- l'hepatopancreas de la crevette caramote *Penaeus trisulcatus* Leach. *Bull. Soc. Sci. Nat. Tunisie* **2**, 13.
- Herdman, W. A. (1904). Recent investigations on pearls in shellfish. *Proc. Trans. Liverpool Biol. Soc.* **17**, 88–97.
- Herdman, W. A., and Hornell, J. (1906). Pearl production, In "Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar" (W. A. Herdman, ed.), Part V, pp. 1–42. R. Soc. London, London.
- Herrick, F. H. (1895). The American lobster; a study of its habits and development. *Bull. U.S. Fish Comm.* **15**, 1–252.
- Hopkins, S. H. (1947). The nemertean *Carcinonemertes* as an indicator of the spawning history of the host, *Callinectes sapidus*. *J. Parasitol.* **33**, 146–150.
- Hopkins, S. H. (1954a). The American species of trematode confused with *Bucephalus* (*Bucephalopsis*) *haimeanus*. *Parasitology*, **44**, 353–370.
- Hopkins, S. H. (1954b). *Cercaria brachidontis* n. sp. from the hooked mussel in Louisiana. *J. Parasitol.* **40**, 29–31.
- Hopkins, S. H. (1957a). Interrelations of organisms. B. Parasitism. In "Treatise on Marine Ecology and Paleoecology" (J. W. Hedgpeth, ed.), Vol. 1, Chap. 15. Geol. Soc. Am., New York.
- Hopkins, S. H. (1957b). Our present knowledge of the oyster parasite "*Bucephalus*." *Proc. Natl. Shellfish. Assoc.* **47**, 58–61.
- Hopkins, S. H. (1958). Trematode parasites of *Donax variabilis* at Mustang Island, Texas. *Publ. Inst. Mar. Sci., Univ. Texas* **5**, 301–311.
- Hoshina, T., and Ogino, C. (1951). Studien ueber *Gymnophalloides tokiensis* Fujita, 1925. I. Ueber die Einwirkung der larvalen Trematoda auf die chemische Komponente und das Wachstum von *Ostrea gigas* Thunberg. *J. Tokyo Univ. Fish.* **38**, 355–350.
- Howell, M. (1966). A contribution to the life history of *Bucephalus longicornutus* (Manter, 1954). *Zool. Publ. Victoria Univ. N.Z.* **40**, 42 pp.
- Howell, M. (1967). The trematode, *Bucephalus longicornutus* (Manter, 1954) in the New Zealand mud-oyster, *Ostrea lutaria*. *Trans. R. Soc. N.Z.* **8**, 221–237.
- Hutton, R. F. (1952). Schistosome cercariae as the probable cause of seabather's eruption. *Bull. Mar. Sci. Gulf Caribb.* **2**, 346–359.
- Hutton, R. F. (1953). *Cercaria reesi* n. sp., a new furcocercous larva from Plymouth. *J. Mar. Biol. Assoc. U.K.* **31**, 581–585.
- Hutton, R. F. (1960). Marine dermatosis. Notes on "seabather's eruption" with *Creseis acicula* Rang (Mollusca: Pteropoda) as the cause of a particular type of sea sting along the west coast of Florida. *Arch. Dermatol.* **82**, 951–956.
- Hutton, R. F. (1964). A second list of parasites from marine and coastal animals of Florida. *Trans. Am. Microsc. Soc.* **83**, 439–447.
- Hutton, R. F., and Sogandares-Bernal, F. (1959). Notes on the distribution of the leech, *Myzobdella lugubris* Leidy, and its association with mortality of the blue crab, *Callinectes sapidus* Rathbun. *J. Parasitol.* **45**, 384, 404, 430.
- Hutton, R. F., Sogandares-Bernal, F., Eldred, B., Ingle, R. M., and Woodburn, K. D. (1959). Investigations on the parasites and diseases of salt-water shrimps (Penaeidae) of sports and commercial importance to Florida. *Fla., State Board Conserv., Mar. Lab., Tech. Ser.* **26** 1–38.
- James, B. L. (1964). The life cycle of *Parvatrema homoeotecnium* sp. nov. (Trematoda: Digenea) and a review of the family Gymnophallidae Morozov, 1955. *Parasitology*, **54**, 1–41.
- Jameson, H. L. (1902). On the origin of pearls. *Proc. Zool. Soc. London* pp. 140–165.
- Jameson, H. L. (1912). Studies on pearl oysters and pearls. (1) The structure of the shell and pearls of the Ceylon pearl oyster (*Margaritifera vulgaris* Schumacher) with an

- examination of the cestode theory of pearl formation. *Proc. Zool. Soc. London* pp. 260–358.
- Jameson, H. L., and Nicoll, W. R. (1913). On some parasites of the scoter duck (*Oedemia nigra*) and their relation to the pearl-inducing trematode in the edible mussel (*Mytilus edulis*). *Proc. Zool. Soc. London* pp. 53–63.
- Johnson, P. T. (1968). Population crashes in the bean clam, *Donax gouldi*, and their significance to the study of mass mortality in other marine invertebrates. *J. Invertebr. Pathol.* **12**, 349–358.
- Johnston, T. H., and Mawson, P. M. (1945). Some parasitic nematodes from South Australian marine fish. *Trans. R. Soc. S. Aust.* **69**, 114–117.
- Kannevorff, B., and Christensen, A. M. (1966). *Kronborgia caridicola* sp. nov., an endo-parasitic turbellarian from North Atlantic shrimps. *Ophelia* **3**, 65–80.
- Katksky, S. C., and Warner, R. W. (1969). Infestation of the rough-sided littleneck clam, *Protothaca laciniata*, in Morro Bay, California, with larval cestodes (*Echeneibothrium* sp.). *J. Invertebr. Pathol.* **13**, 125–128.
- Katksky, S. C., Warner, R. W., and Poole, R. L. (1969). On the occurrence of larval cestodes in the Washington clam, *Saxidomus nuttalli*, and the gaper clam, *Tresus nuttalli*, from Drakes Estero, California. *Calif. Fish Game* **55**, 317–322.
- Kern, F. G., III (1977). Distribution and prevalence of larval anisakid nematodes in surf clams from the middle Atlantic coast. *Proc. Annu. Meet. Am. Soc. Parasitol.* **52**, 53 (abstract).
- Ko, R. C. (1975). *Echinocephalus sinensis* n. sp. (Nematoda: Gnathostomatidae) from the ray (*Aetabatus flagellum*) in Hong Kong, southern China. *Can. J. Zool.* **53**, 490–500.
- Ko, R. C. (1976). Experimental infection of mammals with larval *Echinocephalus sinensis* (Nematoda: Gnathostomatidae) from oysters (*Crassostrea gigas*). *Can. J. Zool.* **54**, 597–609.
- Ko, R. C., Morton, B. and Wong, P. S. (1975). Prevalence and histopathology of *Echinocephalus sinensis* (Nematoda: Gnathostomatidae) in natural and experimental hosts. *Can. J. Zool.* **53**, 550–559.
- Kruse, D. N. (1959). Parasites of the commercial shrimps *Penaeus aztecus* Ives, *P. duorarum* Burkenroad, and *P. setiferus* (Linnaeus). *Tulane Stud. Zool.* **7**, 123–144.
- Lacaze-Duthiers, F. J. H. (1854). Mémoire sur le bucephale Haime (*Bucephalus haimeanus*) helminth parasite des huîtres et des bucardes. *Ann. Sci. Nat., Zool.* **1**, 294–302.
- Lambert, T. C., and Farley, J. (1968). The effect of parasitism by the trematode *Cryptocotyle lingua* (Creplin) on zonation and winter migration of the common periwinkle, *Littorina littorea* (L.). *Can. J. Zool.* **46**, 1139–1147.
- Lauckner, G. (1983). Diseases of Mollusca: Bivalvia, In "Diseases of Marine Animals" (O. Kinne, ed.), Vol. 2, pp. 477–961. Biol. Anstalt Helgol., Hamburg, FRG.
- Lester, R. J. G., Blair, D., and Heald, D. (1980). Nematodes from scallops and turtles from Shark Bay, western Australia. *Aust. J. Mar. Freshwater Res.* **31**, 713–717.
- Levinson, G. M. R. (1881). Bidrag til Kundstab om Grønlands Trematodfauna. *Overs. K. Danske Vidensk. Selsk. Forh.* **23**, 52–84.
- Lichtenfels, J. R., Kern, F. G., III, Zwerner, D. E., Bier, J. W., and Madden, P. A. (1976). Anisakid nematode in shellfish of Atlantic continental shelf of North America. *Trans. Am. Microsc. Soc.* **95**, 265–266 (abstract).
- Lichtenfels, J. R., Bier, J. W., and Madden, P. A. (1978). Larval anisakid (*Sulcascaris*) nematodes from Atlantic molluscs with marine turtles as definitive hosts. *Trans. Am. Microsc. Soc.* **97**, 199–207.
- Lichtenfels, J. R., Sawyer, T. K., and Miller, G. C. (1980). New hosts for larval *Sulcascaris* sp. (Nematoda, Anisakidae) and prevalence in the calico scallop (*Argopecten gibbus*). *Trans. Am. Microsc. Soc.* **99**, 448–451.

- Linton, E. (1940). Trematodes from fishes mainly from the Woods Hole region, Massachusetts. *Proc. U.S. Natl. Mus.* **88**, 1–172.
- Little, J. W., Hopkins, S. H., and Schlicht, F. G. (1966). *Acanthoparyphium spinulosum* (Trematoda: Echinostomatidae) in oysters at Port Isabel, Texas. *J. Parasitol.* **52**, 663.
- MacGinitie, G. E., and MacGinitie, N. (1949). "Natural History of Marine Animals." McGraw-Hill, New York.
- Machkevsky, V. K., and Parukhin, A. M. (1981). On the role of trematodes of the genus *Proctoeces* Odhner, 1911 in certain littoral biocoenoses of the Black Sea. *Vestn. Zool.* **1**, 59–61. (in Russian; English summary).
- MacKay, D. C. (1942). The Pacific edible crab, *Cancer magister*. *Bull. Fish. Res. Board Can.* **62**, 1–32.
- MacKenzie, K. (1963). *Stichocotyle nephropis* Cunningham, 1887 (Trematoda) in Scottish waters. *Ann. Mag. Nat. Hist.* **6**, 505–506.
- Mackin, J. G., and Loesch, H. (1955). A haplosporidian hyperparasite of oysters. *Proc. Natl. Shellfish. Assoc.* **45**, 182–183.
- Manter, H. W. (1940). Gasterostomes (Trematoda) of Tortugas, Florida. *Carnegie Ins. Washington Publ.* **534**, 1–19.
- Margolis, L., and Butler, T. H. (1954). An unusual and heavy infection of a prawn, *Pandalus borealis* Kroyer, by a nematode, *Contracaecum* sp. *J. Parasitol.* **40**, 649–655.
- Martinez, J.-C. (1973). La progénèse dans le cycle évolutif de *Proctoeces maculatus* (Loss, 1901) Odhner, 1911 (Trematoda, Fellodistomatidae). *Bull. Soc. Hist. Nat. Afr. N.* **64**, 39–46.
- McGrady, J. (1874). Observations on the food and reproductive organs of *Ostrea virginica*, with some account of *Bucephalus cuculus* nov. sp. *Proc. Boston Soc. Nat. Hist.* **16**, 170–192.
- Menzel, R. W., and Hopkins, S. H. (1955a). Effects of two parasites on the growth of oysters. *Proc. Natl. Shellfish. Assoc.* **45**, 184–186.
- Menzel, R. W., and Hopkins, S. H. (1955b). The growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus*. *J. Parasitol.* **41**, 333–342.
- Millar, R. H. (1963). Oysters killed by trematode parasites. *Nature (London)* **197**, 616.
- Millemann, R. E. (1951). *Echinocephalus pseudouncinatus* n. sp., a nematode parasite of the abalone. *J. Parasitol.* **37**, 435–439.
- Millemann, R. E. (1963). Studies on the taxonomy and life history of echinocephalid worms (Nematoda: Spiruroidea) with a complete description of *Echinocephalus pseudouncinatus* Millemann 1951. *J. Parasitol.* **49**, 754–764.
- Montreuil, P. (1954). Parasitological investigations. *Rapp. Annu. Stn. Biol. Mar. Dep. Pêches* 1953 **50** (Appendix 5), 69–73.
- Moore, J. P. (1946). The anatomy and systematic position of *Myzobdella lugubris* Leidy (Hirudinea). *Not. Nat. Acad. Nat. Sci. Philadelphia* **184**, 1–12.
- Moore, J. P., and Meyer, M. C. (1951). Leeches (Hirudinea) from Alaskan and adjacent waters. *Wasmann J. Biol.* **9**, 11–77.
- Nickerson, W. S. (1894). On *Stichocotyle nephropis* Cunningham, a parasite of the American lobster. *Zool. Jahrb., Abt. Anat.* **8**, 447–480.
- Odhner, T. (1898). Über die geschlechtsreife Form von *Stichocotyle nephropis* Cunningham. *Zool. Anz.* **21**, 509–513.
- Odhner, T. (1905). Die Trematoden des arktischen Gebietes. In "Fauna Artica" (F. Römer and F. R. Schaudinn, eds.), Vol. 4, p. 291. Fischer, Jena, FRG.
- Odhner, T. (1910). *Stichocotyle nephropis* J. T. Cunningham ein aberranter Trematode der Digenenfamilie Aspidogastriidae. *K. Sven. Vetenskapsakad. Handl.* **45**, 1–16.
- Oka, A. (1927). Sur la morphologie externe de *Carcinobdella kanibir*. *Proc. Imp. Acad. (Tokyo)* **3**, 171–174.

- Overstreet, R. M. (1973). Parasites of some penaeid shrimps with emphasis on reared hosts. *Aquaculture* **2**, 105–140.
- Overstreet, R. M. (1978). Marine maladies? Worms, germs, and other symbionts from the northern Gulf of Mexico. *Miss.—Ala. Sea Grant Consortium Publ.* **78-021**, 140 pp.
- Overstreet, R. M. (1983). Metazoan symbionts of crustaceans. In "The Biology of Crustacea: Vol. 6 Pathobiology" (A. J. Provenzano, ed.), pp. 155–250. Academic Press, New York.
- Ozaki, Y., and Ishibashi, C. (1934). Notes on the cercariae of the pearl oyster. *Proc. Imp. Acad. (Tokyo)* **10**, 439–441.
- Palombi, A. (1924). Le cercarie del genere *Gymnophallus* Odhner dei mitili. *Pubbl. Stn. Zool. Napoli* **5**, 137–152.
- Payne, W. L., Gerding, T. A., Dent, R. G., Bier, J. W., and Jackson, G. J. (1980). Survey of the U.S. Atlantic coast surf clam, *Spisula solidissima*, and clam products for anisakine nematodes and hyperparasitic Protozoa. *J. Parasitol.* **66**, 150–153.
- Pelseneer, P. (1896). Un trématode produisant la castration parasitaire chez *Donax trunculus*. *Bull. Sci. Fr. Belg.* **27**, 357–363.
- Pelseneer, P. (1906). Trématodes parasites de mollusques marins. *Bull. Sci. Fr. Belg.* **40**, 161–186.
- Pelseneer, P. (1928). Les parasites des mollusques et les mollusques parasites. *Bull. Soc. Zool. Fr.* **53**, 158–189.
- Penner, L. R. (1942). Studies on dermatitis-producing schistosomes in Eastern Massachusetts, with emphasis on the status of *Schistosomatum pathlopticum* Tanabe, 1923. *J. Parasitol.* **28**, 103–116.
- Penner, L. R. (1950). *Cercaria littorinalinae* sp. nov., a dermatitis-producing schistosome larva from the marine snail, *Littorina planaxis* Philippi. *J. Parasitol.* **36**, 466–472.
- Perkins, F. O. (1971). Sporulation in the trematode hyperparasite *Urosporidium crescens* De Turk, 1940 (Haplosporida: Haplosporidiidae)—an electron microscope study. *J. Parasitol.* **57**, 9–23.
- Perkins, F. O., Zwerner, D. E., and Dias, R. K. (1975). The hyperparasite, *Urosporidium spisuli* sp. n. (Haplosporea), and its effects on the surf clam industry. *J. Parasitol.* **61**, 944–949.
- Ragan, J. G., and Aldrich, D. V. (1972). Infection of brown shrimp, *Penaeus aztecus* Ives by *Prochristianella penaei* Kruse (Cestoda: Trypanorhyncha) in southeast Louisiana bays. *Trans. Am. Fish. Soc.* **101**, 226–238.
- Rees, F. G. (1937). The anatomy and encystment of *Cercaria purpurae* Lebour, 1911. *Proc. Zool. Soc. London, Ser. B* **107**, 65–73.
- Rees, F. G. (1939). *Cercaria strigata* Lebour from *Cardium edule* and *Tellina tenuis*. *Parasitology* **31**, 458–463.
- Rees, F. G. (1940). Studies on the germ-cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. Part II. Structure of the miracidium and germinal development in the larval stages. *Parasitology* **32**, 372–391.
- Rifkin, E., and Cheng, T. C. (1968). The origin, structure, and histochemical characterization of encapsulating cysts in the oyster *Crassostrea virginica* parasitized by the cestode *Tylocephalum* sp. *J. Invertebr. Pathol.* **10**, 54–64.
- Rifkin, E., Cheng, T. C., and Hohl, H. R. (1969). An electron-microscope study of the constituents of encapsulating cysts in the American oyster, *Crassostrea virginica*, formed in response to *Tylocephalum* metacestodes. *J. Invertebr. Pathol.* **14**, 211–226.
- Robson, R. M., and Williams, I. C. (1971). Relationships of some species of Digenea with the marine prosobranch *Littorina littorea* (L.). II. The effect of larval Digenea on the reproductive biology of *L. littorea*. *J. Helminthol.* **45**, 145–159.
- Sakaguchi, S. (1962). Studies on a trematode parasitic on pearl oysters. I. On the encysta-

- tion of cercaria, *Bucephalus margaritae*. *Kokuritsu Shinju Kenkyusho Hokoku* **8**, 1060–1063 (in Japanese).
- Sakaguchi, S. (1964). Studies on a trematode parasite of pearl oysters. II. Its effects on pearl oyster as the first intermediate host. *Kokuritsu Shinju Kenkyusho Hokoku* **9**, 1161–1169 (in Japanese; English summary.)
- Sakaguchi, S. (1965). Studies on a trematode parasite of the pearl oyster (*Pinctada martensii*). V. Development of its cercaria in the first intermediate host. *Kokuritsu Shinju Kenkyusho Hokoku* **10**, 1244–1253 (in Japanese; English summary).
- Sakaguchi, S. (1966a). Studies on a trematode parasite of the pearl oyster *Pinctada martensii*. III. The metacercaria obtained from the second intermediate host artificially infected with the cercaria, *Bucephalus margaritae*. *Nippon Suisan Gakkaishi* **32**, 312–315 (in Japanese; English summary).
- Sakaguchi, S. (1966b). Studies on the trematode parasite of the pearl oyster, *Pinctada martensii*. IV. On the Trematoda of genus *Bucephalus* found in the fishes, *Caranx sexfasciatus* and *C. ignobilis*. *Nippon Suisan Gakkaishi* **32**, 316–321 (in Japanese, English summary).
- Sakaguchi, S. (1967). Studies on a trematode parasite of the pearl oyster, *Pinctada martensii*. IV. Artificial infection with the encysted metacercaria to the final host. *Kokuritsu Shinju Kenkyusho Hokoku* **12**, 1445–1454 (in Japanese; English summary).
- Sakaguchi, S. (1968). Studies on the life-history of the trematode parasitic in the pearl oyster, *Pinctada fucata*, and on the hindrance for pearl culture. *Kokuritsu Shinju Kenkyusho Hokoku* **13**, 1635–1688 (in Japanese; English summary).
- Sakaguchi, S., Hoshina, T., and Minami, T. (1970a). Studies on a trematode genus, *Proctoeces*, parasitic in the pearl oyster, *Pinctada fucata*. I. On the morphology, parasitic status and the distribution of pearl oysters infected with the parasite. *Kokuritsu Shinju Kenkyusho Hokoku* **15**, 1931–1938 (in Japanese; English summary).
- Sakaguchi, S., Hosina, T., and Minami, T. (1970a). Studies on a trematode genus, *Proctoeces*, parasitic in the pearl oyster, *Pinctada fucata*. I. On the morphology, parasitic status and the distribution of pearl oysters infected with the parasite. *Kokuritsu Shinju Kenkyusho Hokoku* **15**, 1931–1938 (in Japanese; English summary).
- Sakaguchi, S., Minami, T., and Yamamura, Y. (1970b). Studies on a trematode genus, *Proctoeces*, parasitic in the pearl oyster, *Pinctada fucata*. II. Experimental infection with the metacercaria to the final host. *Kokuritsu Shinju Kenkyusho Hokoku* **15**, 1939–1947 (in Japanese; English summary).
- Sawyer, T. K., Rosenfield, A., Perkins, F. O., Zwerner, D. E., Dias, R. K., Lichtenfels, J. R., Madden, P. A., Jackson, G. J., Bier, J. W., Payne, W. L., and Miller, G. C. (1983). Identification of parasitic nematode larvae, *Sulcascaris sulcata*, in the calico scallop, *Argopecten gibbus* (Linnaeus) and the surf clam, *Spisula solidissima*. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **182**, 140–143.
- Schmidt, G. D., and MacLean, S. A. (1978). *Polymorphus (Protilicolis) major* Lundstrom 1942 juveniles in rock crabs, *Cancer irroratus*, from Maine. *J. Parasitol.* **64**, 953–954.
- Shields, J., and Kuris, A. M. (1983). An epidemic of *Carcinonemertes epialti* in an unfished population of *Hemigrapsus oregonensis*. *Am. Zool.* **23**, 924.
- Shipley, A. E., and Hornell, J. (1904). The parasites of the pearl oyster. In "Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar" (W. A. Herdman, ed.), Part II, pp. 77–106. R. Soc. London, London.
- Shipley, A. E., and Hornell, J. (1906). Report on the cestode and nematode parasites from the marine fishes of Ceylon. In "Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar" (W. A. Herdman, ed.), Part V, pp. 43–96. R. Soc. London, London.

- Shuster, C. N., Jr., and Hillman, R. E. (1963). Comments on "Microecological Factors in Oyster Epizootics" by Marshall Laird. *Chesapeake Sci.* **4**, 101–103.
- Sindermann, C. J. (1960). Ecological studies of marine dermatitis-producing schistosome larvae in northern New England. *Ecology* **41**, 785–790.
- Sindermann, C. J. (1965). Effects of environment on several diseases of herring from the western North Atlantic. *Int. Comm. N.W. Atl. Fish., Spec. Publ.* **6**, 603–610.
- Sindermann, C. J., and Farrin, A. E. (1962). Ecological studies of *Cryptocotyle lingua* (Trematoda: Heterophyidae) whose larvae cause "pigment spots" of marine fish. *Ecology* **43**, 69–75.
- Sindermann, C. J., and Gibbs, R. F. (1953). A dermatitis-producing schistosome which causes "clam diggers' itch" along the central Maine coast. *Maine Dep. Sea Shore Fish., Res. Bull.* **12**, 1–20.
- Sindermann, C. J., and Rosenfield, A. (1967). Principal diseases of commercial important marine bivalve Mollusca and Crustacea. *U.S. Fish Wildl. Serv., Fish. Bull.* **66**, 335–385.
- Sivalingham, S. (1962). Bibliography on pearl oysters. *Fish. Res. Stn. Dep. Fish., Ceylon* **13**, 1–21.
- Sogandares-Bernal, F., and Hutton, R. F. (1959). The identity of metacercaria B reported from the pink shrimp, *Penaeus duorarum* Burkenroad, by Woodburn et al. in 1957. *J. Parasitol.* **45**, 362, 378, 384.
- Southwell, T. (1924). The pearl-inducing worm in the Ceylon pearl oyster. *Ann. Trop. Med. Parasitol.* **18**, 37–53.
- Sparks, A. K. (1963). Infection of *Crassostrea virginica* (Gmelin) from Hawaii with a larval tapeworm, *Tylocephalum*. *J. Insect Pathol.* **5**, 284–288.
- Sparks, A. K. (1985). "Synopsis of Invertebrate Pathology Exclusive of Insects," 423 pp. Elsevier, Amsterdam.
- Sparks, A. K., and Chew, K. K. (1966). Gross infestation of the little neck clam, *Venerupis staminea*, with a larval cestode (*Echeneibothrium* sp.). *J. Invertebr. Pathol.* **8**, 413–416.
- Sparks, A. K., and Hibbits, J. (1981). A trematode metacercaria encysted in the nerves of the Dungeness crab, *Cancer magister*. *J. Invertebr. Pathol.* **38**, 88–93.
- Sparks, A. K., and Mackin, J. G. (1957). A larval trypanorhynchid cestode from commercial shrimp. *Tex. J. Sci.* **9**, 475–476.
- Sprague, V. (1964). *Nosema dollfusi* n. sp. (Microsporidia, Nosematidae), a hyperparasite of *Bucephalus cuculus* in *Crassostrea virginica*. *J. Protozool.* **11**, 381–385.
- Sprent, J. F. A. (1977). Ascaridoid nematodes of amphibians and reptiles: *Sulcascaris*. *J. Helminthol.* **51**, 379–387.
- Squires, H. J. (1957). Squid, *Illex illecebrosus* (LeSueur) in the Newfoundland fishing area. *J. Fish. Res. Board Can.* **14**, 693–728.
- Stafford, J. (1912). On the fauna of the Atlantic coast of Canada. Third Report—Gaspe, 1905–1906. *Contrib. Can. Biol. Fish.* **1906–1912**, 45–67.
- Stunkard, H. W. (1938). The morphology and life history of the trematode *Himasthla quissetensis* (Miller and Northup, 1926). *Biol. Bull. (Woods Hole, Mass.)* **75**, 145–164.
- Stunkard, H. W. (1957). The morphology and life history of the digenetic trematode, *Microphallus similis* (Jagerskiold, 1900) Baer, 1943. *Biol. Bull. (Woods Hole, Mass.)* **112**, 254–266.
- Stunkard, H. W. (1960). Further studies on the trematode genus *Himasthla* with descriptions of *H. mcintoshi* n. sp., *H. piscicola* n. sp., and stages in the life-history of *H. compacta* n. sp. *Biol. Bull. (Woods Hole, Mass.)* **119**, 529–549.
- Stunkard, H. W., and Hinchliffe, M. G. (1952). The morphology and life history of *Microbilharzia variglandis* (Miller and Northup, 1926) Stunkard and Hinchliffe, 1951, avian blood flukes whose larvae cause "swimmer's itch" of ocean beaches. *J. Parasitol.* **38**, 248–265.

- Stunkard, H. W., and Shaw, C. R. (1931). The effect of dilution of sea water on the activity and longevity of certain marine cercariae with descriptions of two new species. *Biol. Bull. (Woods Hole, Mass.)* **61**, 242–271.
- Stunkard, H. W., and Uzmann, J. R. (1958). Studies on digenetic trematodes of the genera *Gymnophallus* and *Parvatremia*. *Biol. Bull. (Woods Hole, Mass.)* **115**, 275–302.
- Stunkard, H. W., and Uzmann, J. R. (1959). The life cycle of the digenetic trematode, *Proctoeces maculatus* (Looss, 1901) Odhner, 1911 (syn. *P. subtenuis* (Linton 1907) Hanson, 1950), and description of *Cercaria adranocerca* n. sp. *Biol. Bull. (Woods Hole, Mass.)* **116**, 184–193.
- Sund, O. (1914). Beretning om anlaeg av statens hummeravlsstation og driften i 1913. *Aarsberet. Norg. Fisk.* **4**, 525–532.
- Sund, O. (1915). Statens hummeravlsstation, Korshavn. *Aarsberet. Norg. Fisk.* **2**, 176–181.
- Tennent, D. H. (1906). A study of the life history of *Bucephalus haimeanus*; a parasite of the oyster. *Microsc. Sci.* **49**, 635–690.
- Timon-David, J. (1949). Sur un trématode parasite des crabes en Méditerranée. *Ann. Parasitol. Hum. Comp.* **24**, 25–28.
- Uhazy, L. S., Aiken, D. E., and Campbell, A. (1985). Morphology and systematics of the nemertean *Pseudocarcinonemertes homari* (Hoplonemertea: Monostilifera) from the American lobster, *Homarus americanus*. *Can. J. Fish. Aquat. Sci.* **42**, 342–350.
- Uspenskaya, A. B. (1953). The life cycles of nematodes of the genus *Ascarophis* vanBeneden (Nematoda: Spirurata). *Zool. Zh.* **32**, 828–832 (in Russian).
- Uzmann, J. R. (1951). Record of the larval trematode *Himasthla quissetensis* (Miller and Northup, 1926) Stunkard, 1934 in the clam, *Mya arenaria*. *J. Parasitol.* **37**, 327–328.
- Uzmann, J. R. (1952). *Cercaria myae* sp. nov., a fork-tailed larva from the marine bivalve, *Mya arenaria*. *J. Parasitol.* **38**, 161–164.
- Uzmann, J. R. (1953). *Cercaria milfordensis* nov. sp., a microcercous trematode larva from the marine bivalve, *Mytilus edulis* L. with special reference to its effect on the host. *J. Parasitol.* **39**, 445–451.
- Uzmann, J. R. (1967a). Juvenile *Ascarophis* (Nematoda: Spiruroidea) in the American lobster, *Homarus americanus*. *J. Parasitol.* **53**, 218.
- Uzmann, J. R. (1967b). *Histriobdella homari* (Annelida: Polychaeta) in the American lobster, *Homarus americanus*. *J. Parasitol.* **53**, 210–211.
- Uzmann, J. R. (1970). Use of parasites in identifying lobster stocks. *J. Parasitol.* **56**, 349 (abstract).
- Van Engel, W. A., Harris, R. E., Jr., and Zwerner, D. E. (1986). Occurrence of some parasites and a commensal in the American lobster, *Homarus americanus*, from the Mid-Atlantic Bight. *Fish. Bull.* **84**, 197–200.
- Villella, J. B., Iversen, E. S., and Sindermann, C. J. (1970). Comparison of the parasites of pond-reared and wild pink shrimp (*Penaeus duorarum* Burkenroad) in South Florida. *Trans. Am. Fish. Soc.* **99**, 789–794.
- Ward, J. W. (1962). Helminth parasites of some marine animals, with special reference to those from the yellow-fin tuna, *Thunnus albacares* (Bonnaterre). *J. Parasitol.* **48**, 155.
- Warner, R. W., and Katkasky, S. C. (1969). Infestation of the clam *Protothaca staminea* by two species of tetraphyllidian cestodes (*Echeneibothrium* spp.). *J. Invertebr. Pathol.* **13**, 129–133.
- Wickham, D. E. (1979). Predation by the nemertean *Carcinonemertes errans* on the eggs of the Dungeness crab *Cancer magister*. *Mar. Biol. (Berlin)* **55**, 45–53.
- Wickham, D. E. (1980). Aspects of the life history of *Carcinonemertes errans* (Nemertea: Carcinonemertidae), an egg predator of the crab *Cancer magister*. *Biol. Bull. (Woods Hole, Mass.)* **159**, 247–257.

- Wickham, D. E. (1986). Epizootic infestations by nemertean brood parasites on commercially important crustaceans. *Can. J. Fish. Aquat. Sci.* **43**, 2295–2302.
- Wickham, D. E., Beau, S. F., and Kuris, A. M. (1985). Preliminary report on egg mortality in Alaskan king crabs caused by the egg predator *Carcinonemertes*. *Proc. Int. King Crab Symp. Ser., Alaska Sea Grant Rep.* **85-12**, 265–270.
- Williams, I. C., and Ellis, C. (1976). Larval digenea in Shetland. *Glasgow Nat.* **19**, 307–315.
- Wolf, P. H. (1976). Occurrence of larval stages of *Tylocephalum* (Cestoda: Lecanicephaloidea) in two oyster species from northern Australia. *J. Invertebr. Pathol.* **27**, 129–131.
- Woodburn, K. D., Eldred, B., Clark, E., Hutton, R. F., and Ingle, R. M. (1957). The live bait shrimp industry of the west coast of Florida (Cedar Key to Naples). *Fla., State Board Conserv., Tech. Ser.* **21**, 1–33.
- Wright, C. A. (1966). The pathogenesis of helminths in the Mollusca. *Helminthol. Abstr.* **35**, 207–224.
- Yamaguti, S. (1938). Studies on the helminth fauna of Japan. Part 21. Trematodes of Fishes, Vol. IV, pp 1–139. Kyoto, Japan.
- Yamaguti, S. (1938). Studies on the helminth fauna of Japan. Part 21. In "Trematodes of Fishes," Vol. IV, pp 1–139. Kyoto, Japan.
- Young, R. T. (1953). *Postmonorchis donacis*, a new species of monorchid trematode from the Pacific coast, and its life history. *J. Wash. Acad. Sci.* **43**, 88–93.

7

Parasitic Crustaceans

I. INTRODUCTION

Crustacean parasites of fish, as examined in Volume 1 of this edition, can on occasion be severely pathogenic (some of the tissue-invading copepods, for example). The same comment could be made in this volume about crustacean parasites of molluscs and crustaceans: that a few may be pathogenic, but the overall effects are relatively minor when compared with microbial agents.

Nevertheless, interesting and unusual organisms are included in this category of crustacean parasites of shellfish. The bivalve molluscs harbor copepod parasites of the gut, notably members of the genus *Mytilicola*. Crabs are favorite hosts for rhizocephalan barnacles and epicaridean isopods, both of which have reputations as “parasitic castrators” of their hosts.

Effects of crustacean parasites on invertebrate hosts are usually classified as “sublethal”—consisting of destruction of gonads, absorption of nutrients, induction of structural abnormalities, erosion of gills, modification of physiological functions, occlusion of ducts, and a variety of other pathologies. Direct association of crustacean disease agents with host mortalities is unusual, although not unknown.

This chapter, following the plan of preceding ones, examines the crustacean parasites of molluscs first, and then considers crustacean parasites of crustaceans, attempting to emphasize those agents that cause disease in economic species.

II. DISEASES OF MOLLUSCS CAUSED BY PARASITIC CRUSTACEANS

Crustacean parasites are not known as significant pathogens of marine molluscs. A few parasitic copepods and some brachyuran (pinnotherid)

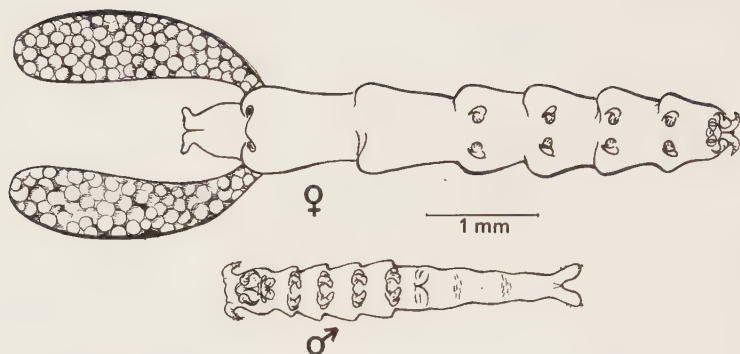


Figure 29. A parasitic copepod, *Mytilicola intestinalis*, from the sea mussel, *Mytilus edulis*.

crabs constitute most of the problem animals—and their effects are slight.

A. Copepods

Among the copepod parasites, *Mytilicola intestinalis* (Fig. 29), first described by Steuer (1902, 1903), has been blamed for widespread mortalities, stunting of growth, and poor condition of sea mussels, *Mytilus edulis*, in Europe and is found occasionally in European oysters (*Ostrea edulis*), but has not been reported to cause significant mortalities in that species (Korringa, 1950, 1959, 1968; Baird *et al.*, 1950; Hepper, 1953, 1955). An enormous European literature has developed because of the presumed (but often poorly documented) pathogenicity of *M. intestinalis* to mussels. The parasite, known as the red worm, or *le cop rouge*, occurs in the host's intestine; as many as 92 individuals have been found in a single mussel, and local prevalences may reach 100% in some mussel-producing areas.

The apparent invasion of North European mussel stocks by *M. intestinalis* and its subsequent spread were reviewed by Korringa (1968) and by Lauckner (1983). The parasite was first described by Steuer (1902) in the intestines of Mediterranean edible mussels, *Mytilus galloprovincialis*, and Pesta (1907) outlined its life history. Korringa (1950, 1953, 1957, 1959) described the relatively sudden appearance of *Mytilicola* in sea mussel stocks of The Netherlands in 1949 and its subsequent spread to many mussel beds during the following decade. The organism is known to have occurred in Mediterranean mussels since the beginning of the 20th century (Monod and Dollfus, 1932) and in 1938 was found near Cuxhaven, Germany, from which it was assumed to have spread

westward to The Netherlands. Korringa and others believed that distribution was aided by mussel-encrusted ships, by the movements of planktonic larvae, and by the transfer of seed mussels from infected areas. *Mytilicola* was also very abundant in localized areas of the English coast in 1946 and remains so today. Korringa stated that the condition of mussels was generally correlated with the intensity of parasitization; mussels with less than five copepods were still healthy, those with five to ten were visibly thinner, and those more heavily infected suffered serious mortalities. According to Meyer-Waarden and Mann (1954b) and Mann (1956), gonad weights of infected individuals were 10–30% lower than those of nonparasitized mussels.

There is some indication, however, that *M. intestinalis* exerts a less severe effect on populations of *M. galloprovincialis* than on those of *M. edulis*, possibly because of longer association and consequent better adaptation of the host and the parasite, as was pointed out by Fleury *et al.* (1951). Hrs-Brenko (1964), for example, found no difference in the condition index of parasitized and non-parasitized mussels (*M. galloprovincialis*) on the Yugoslav Adriatic coast, and Genovese (1959) made similar findings on the Italian coast.

Parasitization of *M. edulis* by *M. intestinalis* during the outbreak period in the 1950s led to poor growth, thin meats, a cream-colored rather than dark brown liver, failure of byssal development, and a dirty red-brown color (Cole and Savage, 1951; Mann, 1951). Reproduction of the parasite was accelerated by warm water, and the many young parasites present in the summer invaded and killed mussels. Deaths occurred among mussels of all sizes, including "seed." Mussels fell from culture racks and died during transport to markets (Brienne, 1964). Density of the mussel beds was believed to directly influence survival and multiplication of the parasite. Infections were light in areas where the mussels were thinly scattered and near the surface of the water. Because of the spread of *Mytilicola* in The Netherlands, an extensive scheme of repeated dredging of natural beds, transfer of lightly infected stocks, and destruction of heavily infected stocks was outlined by Korringa (1959) to create a barrier against further invasion.

Mytilicola in mussel populations grown from floats in Spain was studied by Andreu (1963). He found the infection to be greatest near shore, where tidal currents were weak. Vertical distribution of the parasite in cultured mussels grown on 6-m ropes was uniform in areas of strong currents, but increased with depth where currents were weak. Such findings agree well with those of Hepper (1955), who concluded from field observations that mussels raised from the bottom or in fast-moving water at either end of an estuary were less heavily infected with

Mytilicola than those on the bottom, in slow-moving water, or in the midregions of estuaries. Hepper felt that control of the copepod was possible by using off-bottom culture or by locating culture beds in fast-moving water or at the brackish-water ends of estuaries.

Off-bottom culture, using rafts, was reexamined in relation to *Mytilicola* parasitization by Paul (1983). Prevalences of the parasite in the rías of northern Spain have increased in the 20 years since the studies by Andreu (1963), especially in the inner parts of the rías. This may be a function of an increase in the intensity of cultivation, as was pointed out by Paul (1983). The parasite had little effect on the condition of cultivated mussels, except during the period of recovery from spawning.

Mytilicola intestinalis is known only in Europe, where it has been reported from a number of areas: Germany (Caspers, 1939; Meyer and Mann, 1950, 1952a,b, 1954a,b; Meyer-Waarden and Mann, 1956), The Netherlands (Korringa, 1951a, 1952a,b, 1953, 1968), Belgium (Leloup, 1951, 1960), Scotland, England, and Ireland (Ellenby, 1947; Grainger, 1951; Hockley, 1952; Thomas, 1953; Bolster, 1954; Waugh, 1954, 1966; Davey and Gee, 1976), the north coast of France (Dollfus, 1914; Monod and Dollfus, 1932; Brienne, 1964), the northwest coast of Spain (Andreu, 1960, 1961, 1963), the Mediterranean Sea (Bassedas, 1950; Meyer-Waarden and Mann, 1954b), and the Adriatic Sea (Steuer, 1902; Pesta, 1907; Meyer-Waarden and Mann, 1954b).

At midcentury a major conference was held in Paris to review and discuss problems of parasitization by *Mytilicola*, and the findings were published in 1951 (Cole and Savage, 1951; Dollfus, 1951; Havinga, 1951; Heldt, 1951; Korringa, 1951b; Korringa and Lambert, 1951; Lambert, 1951a,b; Leloup, 1951; Meyer and Mann, 1951). It was agreed that *Mytilicola* constituted a severe threat to the mussel industry of Europe, but the question as to whether the copepod was a direct or indirect cause of death was left undecided. Continuing mortalities associated with the presence of *Mytilicola* (Brienne, 1964; Korringa, 1968), however, indicated a causal relationship, possibly influenced by stresses of spawning, high temperatures, and inadequate food supply. Korringa believed that there was little chance of eradicating *Mytilicola* once it had invaded an area, that it had been a new intruder in many coastal areas, and that humans (rather than other factors) had been responsible for much of the spread of the parasite since 1950.

Studies of *M. intestinalis* in recent decades have cast some doubt on earlier reports of its pathogenicity to mussels. Earlier conclusions were not based on statistical evaluations, searches were not made for other causes of mortalities, and some findings did not demonstrate adverse effects. At present, mortalities have ceased, but prevalences of the para-

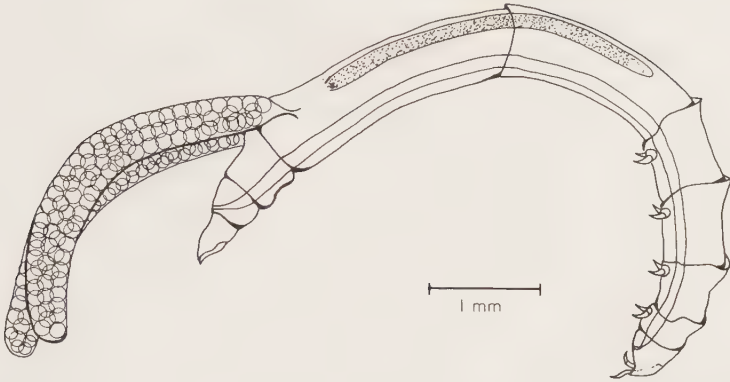


Figure 30. A parasitic copepod, *Mytilicola orientalis*, from the Pacific oyster, *Crassostrea gigas*.

sites have remained high in some areas (Dethlefsen, 1972; Lauckner, 1983). This respite has provided an opportunity for reexamination of aspects of the biology, distribution, and population dynamics of *M. intestinalis* and of its effects on mussels (Williams, 1969). In two studies (on the German and English coasts), a clear relationship of meat content (condition) and parasitization was not found (Dethlefsen, 1975; Gee *et al.*, 1977). A third study (also in England) found no evidence that heavily parasitized mussels were killed (Davey *et al.*, 1978). Epizootic levels of the parasite occur in localized areas, and a slow expansion of its distribution has occurred since 1950 (Lauckner, 1983).

The genus *Mytilicola* is represented best in Pacific molluscs by *Mytilicola orientalis* (Fig. 30), first described by Mori (1935) in the digestive tract of the Pacific oyster, *Crassostrea gigas*, and the mussel, *Mytilus crassitesta*, from Japan. The parasite was transferred to the U. S. West Coast with imports of seed oysters from Japan and was described as *Mytilicola ostreae* by Wilson (1938), who was apparently unaware of Mori's report. The introduced parasite spread along the Pacific coast and to several native species, including the Olympia oyster, *Ostrea lurida*, and the mussels *Mytilus californianus* and *M. edulis* (Chew *et al.*, 1964). Olympia oysters infected with even small numbers of *M. orientalis* had a lower condition index than did uninfected oysters, and a similar relationship was seen in *C. gigas* (Odlaug, 1946; Chew *et al.*, 1965; Katkansky *et al.*, 1967; Katkansky and Warner, 1968). Histopathological changes were demonstrated in the gut epithelium and underlying tissues of *C. gigas* infected with the copepod, but no relationship with mortality was established (Sparks, 1962, 1985).

As an introduced parasite, *M. orientalis* probably has its most severe

effect on Pacific coast mussels (*M. californianus* and, to a lesser degree, *M. edulis*), at least as indicated by prevalences. In one study prevalences were 65% in *M. californianus* and 58% in *M. edulis* (Bernard, 1969).

Mytilicola orientalis was also transported with its normal host, *C. gigas*, to the coasts of France, when that country imported massive numbers of seed and adult oysters from Japan and the west coast of North America during the period 1967–1973. The parasite was first reported in France (in imported *C. gigas*) in 1977 and has since spread to the European or flat oyster, *O. edulis*, and to the sea mussel, *M. edulis*, in at least one location (Arcachon, on the Atlantic coast) (His, 1977, 1979). It is interesting that *C. gigas* seems resistant to infections by the endemic European species of parasitic copepod, *M. intestinalis* (Dare, 1982).

Pacific clams, like oysters and mussels, harbor parasitic copepods. Hoshina and Kuwabara (1959) described *Mytilicola mactrae* from Japanese *Mactra veneriformis* Reeve. About half of the 69 clams sampled were infected. Yamaguti (1939) had described earlier another species of *Mytilicola* from *Brachidontes senhousi*.

Still another species, *Mytilicola porrecta*, occurs in ribbed and recurved mussels (*Modiolus demissus* and *Brachidontes recurvus*) in the Gulf of Mexico. Humes (1954) found as many as 15 individuals per mussel, but no pathology or mortality was indicated. The parasite was also found in hard clams, *Mercenaria mercenaria*, but not in oysters from the Gulf.

To conclude the *Mytilicola* discussion, a copepod parasite of gastropods of the family Trochidae from the French coast was described by Dollfus (1914). Described as *Trochicola enterica*, the copepod localized in the digestive tract, extending almost to the rectum, with egg cases projecting into the pallial cavity of the host. Dollfus found similarities between *T. enterica* and *M. intestinalis*, parasitic in European mussels.

It should be noted that other genera of copepods live in close association with molluscs, especially bivalves, but they usually cause little, if any, pathology, except for some of the gill-inhabiting species, such as *Myicola ostreae*, parasitic on *Crassostrea gigas* and *C. angulata* (His, 1979), and *Pseudomyicola spinosus* from the New Zealand rock oyster, *Crassostrea glomerata* (Dinamani and Gordon, 1974). The latter species also invades the stomach and the digestive diverticula, damaging the epithelium and occluding ducts.

B. Crabs

Turning now to the brachyuran crabs, members of the family Pinnotheridae, often known as pea crabs, inhabit the mantle cavity of bivalve

molluscs. Parasitic only in the broadest definition of the term, pinnotherid crabs have been reported to cause gill and palp lesions, weight loss, and reduced filtering ability in their hosts (Møller-Christensen and McDermott, 1958; Haven, 1959) and for these reasons they deserve some fleeting attention in this book.

Pinnotheres ostreum is a well-known species which inhabits the mantle cavity of the American oyster, *Crassostrea virginica*. Stauber (1945) studied a sudden increase in the abundance of this crab in American oysters of Delaware Bay in 1941. He observed that the crabs robbed the oyster of food and injured the gills, resulting in a weakened condition of the host. Stauber found in 1941 that 90% of the oysters in Delaware Bay harbored four to six crabs in the first parasitic stage. He ascribed unusual mortalities in certain oyster populations to debilitation caused by a high abundance of crabs. In 1942 the abundance of crabs dropped to 25–30% and continued to decline in the following year. Sandoz and Hopkins (1947) observed that “normal” crab parasitization of *C. virginica* (one female per host) caused a decline in condition in Virginia, where as many as 80% of the oysters in particular beds were parasitized.

Another Atlantic coast pinnotherid crab associated with commercial bivalves is *Pinnotheres maculatus*, found in scallops (*Placopecten magellanicus*, *Argopecten irradians*, and *Argopecten gibbus*), clams (*Mya arenaria*), and mussels (*Mytilus edulis* and *Modiolus modiolus*). *Pinnotheres maculatus*, like *P. ostreum*, causes gill and palp damage, especially in *M. edulis* (McDermott, 1962).

On the European coast the most common and most studied pinnotherid is *Pinnotheres pisum*, which occurs in mussels, *M. edulis* and *M. modiolus*, as well as in a variety of other bivalves. Many investigations, some dating back to early in the century, disclosed abnormalities such as emaciation and gill erosion as a consequence of presence of the crabs (Berner, 1952; Seed, 1969).

Pinnotherids occur and have been studied in other parts of the world as well. Oysters (*Saccostrea cucullata*) of India are parasitized by a *Pinnotheres* sp. which was held responsible for changed sex ratios in host populations (Awati and Rai, 1931). Cultivated and wild mussels, *M. edulis*, in Australia are affected by *Pinnotheres hickmani* (Pregenzer, 1978). Mussels (*M. modiolus*, *M. edulis*, and *M. californianus*) from the west coast of North America are hosts to the pinnotherid *Fabia subquadrata* (Pearce, 1966).

Another pinnotherid was described by Poisson (1946) in Madagascar oysters, *Ostrea vitrifacta*; infection was accompanied by the development of a characteristic irritating flavor in the parasitized oyster. Poisson speculated that this flavor might be traced in some way to the coelente-

rate *Sertularia*, which often grows on the shells of oysters that contain *Pinnotheres*. Korringa (1952a) pointed out the striking similarity of this observation with the popular belief in Holland that eating mussels parasitized by *P. pisum* causes "nettle rash."

The copepods and the pinnotherid crabs, then, are the crustacean parasites of molluscs that cause some pathology and possibly some rare mortalities under unfavorable environmental conditions. Any effects on host populations are undoubtedly small, though, when compared with those resulting from microbial infections.

III. DISEASES OF CRUSTACEANS CAUSED BY PARASITIC CRUSTACEANS

Crustaceans are common parasites of marine Crustacea, and some of them can be severely pathogenic to individual members of economically important species, although their total population impact is undoubtedly much lower than that of microbial pathogens. Two crustacean groups—the rhizocephalans (class Cirripedia) and the epicaridean isopods—contain members that can damage their hosts severely, resulting in structural deformation, sterilization, and death in some instances. Additionally, a few copepods may be ectoparasitic on other Crustacea and may cause minor pathology.

A. Rhizocephalans

Many species of crabs, in many parts of the world, are infected by rhizocephalan barnacles. These parasites invade the host's body and cause degeneration of the gonads (Reinhard, 1956). The tumorlike body of the rhizocephalan ramifies throughout the crab, much like a root system (Fig. 31), causing extensive morphological changes; the parasite then produces a saclike "externa," which contains the ovaries and the embryos. This external protrusion of the rhizocephalan is usually located where the host's eggs are normally carried. The infected crab is usually sterilized, secondary sex characters are modified, and molting is often inhibited (Giard, 1888; Potts, 1906, 1915; Smith, 1906; Cantacuzène, 1925; Shiino, 1931; Reinhard, 1950; Ichikawa and Yamagunachi, 1957; Hartnoll, 1967).

Brachyuran, or true, crabs are parasitized by members of the rhizocephalan family Sacculinidae. In U. S. waters the crabs most frequently parasitized are the green crab (*Carcinus maenas*) of the Atlantic coast and the masking crab, *Loxorhynchus grandis*, the kelp crab, *Pugettia producta*, and the black-clawed crab, *Lophopanoepus bellus* of the Pacific

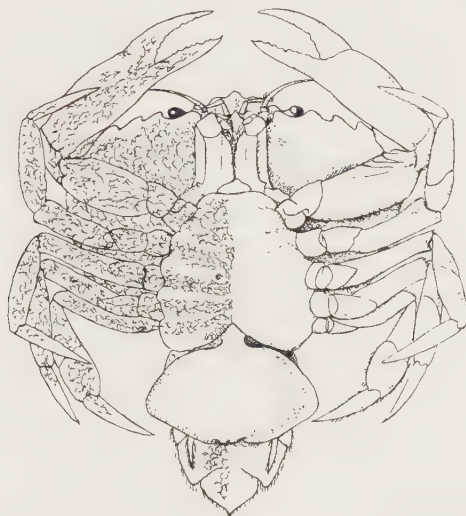


Figure 31. Rootlet system of *Sacculina carcini* in *Carcinus maenas*. (Redrawn, for one half of the crab only, from Bang, 1983.)

coast. Green crabs, *C. maenas*, and swimming crabs, *Macropipus* (*Portunus*) *holsatus*, from the European coast are parasitized by *Sacculina carcini* (Delage, 1884; Boschma, 1928, 1963; Day, 1935; Foxon, 1940; Hubert *et al.*, 1976). *Sacculina carcini* causes morphological changes in immature male hosts, principally a broadening of the abdomen and feminization of some appendages. Rootlets of the parasite's internae invade all organs of the host, including the ganglia and the gonads, with consequent changes in hormone-associated behavior; similar changes can be induced by injections of *Sacculina* extracts into uninfected crabs (Andrieux *et al.*, 1981; Herberts, 1982).

Blue crabs, *Callinectes sapidus*, from the Gulf of Mexico are frequently parasitized by the rhizocephalan *Loxothylacus texanus* (Reinhard, 1950; Hopkins, 1957; Overstreet, 1983). Immature animals are normally infected, and morphological changes in the shape of the abdomen occur (Adkins, 1972). Molting is inhibited, so infected animals are markedly smaller than cohorts (Fig. 32). Epizootics of the rhizocephalan in blue crabs of the Mississippi coastline were reported in 1965 and in 1977; prevalences exceeded 50% (Overstreet, 1978). Since diseased crabs are below market size, the parasite can thereby cause significant economic damage to a fishery during outbreak periods.

A crab of economic importance in Australia and in other parts of the Pacific, the sand crab, *Portunus pelagicus*, is often parasitized by the rhi-



Figure 32. A Blue Crab, *Callinectes sapidus*, Infected with the Rhizocephalan *Loxothylacus texanus* (bottom), compared to a normal egg-bearing female (top). (Courtesy of Dr. R. M. Overstreet.)

zocephalan *Sacculina granifera*. Detailed studies of the organism in Moreton Bay, Queensland, disclosed prevalences of 5–30% in commercial sizes (Phillips and Cannon, 1978). Infected crabs were sterilized, but those with externae participated in normal offshore migrations, along with uninfected egg-bearing females. A significant observation was that invasion of the hepatopancreas by rootlets of the parasite produced a green pigment, which discolored the flesh when crabs were cooked—thereby reducing the market value of the catches. Annual losses to the Australian sand crab fishery due to *S. granifera* have been estimated at 10–15% of its value (Lester, 1978).

Some species of commercial anomuran crabs may also be parasitized by rhizocephalans. King crabs, *Paralithodes platypus* and *Paralithodes camtschatica*, from the Gulf of Alaska are infected by a cosmopolitan parasite, *Briarosaccus callosus* (Boschma and Haynes, 1969; Hawkes *et al.*, 1985). Prevalences of up to 76% were reported in samples of the blue king crab (*P. platypus*) obtained with commercial gear. Atrophy or the apparent absence of gonads was characteristic of parasitized hosts, and much of the hepatopancreas and all other major organs of the crabs were

invaded and replaced by rootlets of *B. callosus* (Hawkes *et al.*, 1985). The same parasite has been reported in golden king crabs, *Lithodes aequispina*, and deep sea king crabs, *Lithodes couesi* (McMullen and Yoshihara, 1970). A second rhizocephalan, possibly a species of *Thompsonia*, has a more localized distribution in Alaskan crabs, reported only in blue king crabs from Kodiak Island (Johnson *et al.*, 1986). Reproductive failure in parasitized females was indicated by empty ovaries or ovaries containing only degenerating gonadal cells. Prevalences of up to 50% were reported, suggesting significant but localized population effects.

Hermit crabs are frequently invaded by members of the rhizocephalan family Peltogastridae. Reinhard (1942), who examined 3092 *Pagurus pubescens* from the Maine coast of the United States, found 13.7% parasitized by *Peltogaster paguri*. The same rhizocephalan occurs on the coast of France, where its host is *Pagurus bernhardus* (Pérez, 1927, 1928, 1931a–c), and in Japan, where it parasitizes four species of hermit crabs (Shiino, 1943). Infection can have significant effects on crab populations, since parasitization usually causes degeneration of host gonads. Pérez (1903, 1929, 1931a), however, found interesting evidence for sterilization and mortalities of *P. paguri* because of hyperparasitization by an epicaridean isopod of the family Cryptoniscidae, *Liriopsis pygmaea*. In some samples from northern France, most of the rhizocephalans were parasitized. Pérez believed that this hyperparasitization was an important control for *Peltogaster* populations. *Liriopsis* has an effect on *Peltogaster* that is similar to that of *Peltogaster* on *Pagurus*: gonadal degeneration.

Rhizocephalans, then, are common and destructive parasites of crabs, inhibiting molting (hence, growth), causing behavioral modifications, sterilizing females, and suppressing the development of male sex characteristics—by mechanical disruption of tissues, by modifying host hormonal secretions, and possibly by producing hormone-mimicking secretions (Bishop and Cannon, 1979; Rubiliani, 1985). A large body of literature on this fascinating group of parasites has accumulated during the past century (see the bibliography by Lawler and Shepard, 1979); some indications of population effects exist, but they lack specificity.

B. Isopods

Epicaridean isopods can also be significant parasites of other crustaceans. Two families are important: the Bopyridae and the Entoniscidae. The Bopyridae are only moderately modified for parasitic existence in the branchial chamber or the abdomen of the host, whereas the Entoniscidae are highly modified and live in the hemocoel of the host, within a sac formed by invagination of the host integument. Modification is

limited to females; the dwarf male retains typical isopod characters. Morphological modification for parasitic existence is similar in some entoniscids to that which occurs in rhizocephalans (Veillet, 1945; Nicol, 1967). Effects on the crab host often include sterilization or suppression of gonadal activity and changes in secondary sexual characteristics of males (Tucker, 1930; Reverberi, 1943, 1952; Reinhard and Buckeridge, 1950). Parasitization of crabs by female entoniscids causes internal deformities, including a reduction in the size of the organs (Atkins, 1933; Reinhard, 1945) and changes in the nervous system (Matsumoto, 1953). The entoniscids are true endoparasites, living in the host's body cavity within a sheath of host response tissue, with an exit pore for the release of larvae located in the host's branchial chamber.

One extensive study of the interactions of an entoniscid parasite, *Portunion conformis*, and its host, the shore crab, *Hemigrapsus oregonensis* (Kuris *et al.*, 1980), disclosed the following:

1. The ovary of the female crab begins to deteriorate soon after establishment of the isopod parasite.
2. The male crab may exhibit feminization of secondary sex characteristics (e.g., width of the abdomen and length of the chelae).
3. The female entoniscid within the host's body cavity is covered by a thin tough cuticle of host origin.
4. Despite this enveloping sheath, developing parasites may be killed by the host's defense mechanisms.
5. Death of the parasite leads to recovery of the host's reproductive capacity, but at a lower level than in normal crabs.

Prevalence of the isopod varied seasonally and geographically in Pacific coast samples of *H. oregonensis*, within a range of 20–91%.

Detailed studies of Epicaridea of crabs have been carried out in several parts of the world. Shiino (1942b), for example, examined the entoniscids parasitizing littoral crabs in the Seto area of Japan. He described seven new species, including *Entoniscus japonicus*, parasitic on the porcellanid crab, *Petrolisthes japonicus*. This was the fifth parasitic crustacean described in *P. japonicus* in the Seto area; Shiino (1933) had previously identified two bopyrids and Boschma (1935), two rhizocephalans. Shiino (1942b) described the entoniscid *Portunion flavidus* as a common parasite of the crab *Pachygrapsus crassipes*, which was also parasitized by two species of *Sacculina*. Shiino (1933, 1934, 1936, 1937, 1939a,b, 1949, 1958, 1964a) has also described bopyrids from other Japanese crabs, as well as those from Palau, California, and Chile (1942a, 1964b,c).

Epicaridean isopods are also well-known parasites of shrimp (Fig.

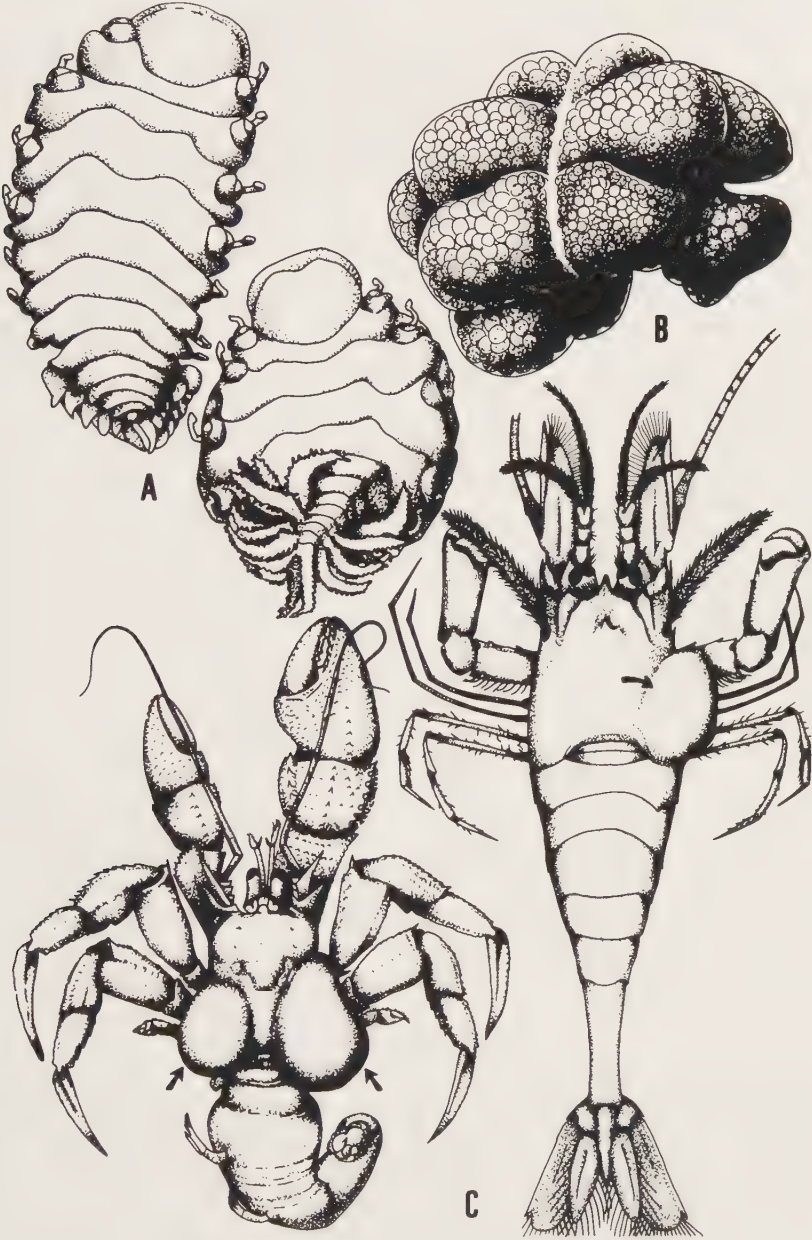


Figure 33. Parasitic isopods and their effects. (A) bopyrid isopods; (B) a gravid female entoniscid isopod; (C) host abnormalities (arrows) resulting from isopod parasitization. [Redrawn from Shiino (1965), Baer (1951), Waterman (1960, 1961), and Williams (1965).]

33). Baer (1951), for example, stated that the abdominal bopyrid, *Hemiarthrus abdominalis*, had been recovered from 20 species of shrimp belonging to the genera *Pandalus* and *Spirontocaris*. J. R. Uzzmann (personal communication, 1967) found *H. abdominalis* on northern pink shrimp from the Gulf of Maine. The parasite has also been reported on *Pandalus borealis* in Greenland (Horsted and Smidt, 1956) but not in Norway or in England (Dahl, 1949; Allen, 1966). *Bopyrus fougerouxii* parasitizes the prawn, *Palaemon serratus* (Brian and Parenzan, 1967; Nicol, 1967), causing suppression of breeding characteristics.

Ricketts and Calvin (1962) described the occurrence of the bopyrid isopod, *Argeia pugettensis*, which caused unilateral protuberances of the carapace of the black-tailed shrimp, *Crago nigricauda*, from the Pacific coast of the United States. Infestation was estimated at 3–5%. Similar large protuberances are seen commonly in the shrimp, *Hippolytina wurdemanni*, of Biscayne Bay, Florida, where 50% of the population was reported to be parasitized and deformed by the isopod *Probopyrus* sp. (Van Arman and Smith, 1970). The presence of the parasite caused atrophy of the underlying gills and musculature, in addition to the exoskeletal abnormality.

Another species of *Probopyrus*, *Probopyrus pandalicola*, is a branchial parasite of numerous species of palaemonid shrimp along the Atlantic seaboard of North America. A recent study indicated the suppression of female gonads by preventing ovarian maturation, but no effects on male gonads (Beck, 1980). The secondary sex characteristics of the females were not affected, but those of the males were.

Japanese red prawns, *Penaeopsis akayebi*, are frequently (up to 70%) infested with another bopyrid, *Epipenaeon japonicus*. Hiraiwa and Sato (1939) found the gonads of parasitized individuals reduced or, in some males, completely atrophied. The presence of the branchial parasite, *Bopyrus squillarum*, on the shrimp, *Leander serrifer*, causes suppression of the ovaries and the breeding characteristics of the pleopods (Yoshida, 1952).

An extensive and fascinating body of literature on rhizocephalan, epicaridean, and other crustacean parasites and hyperparasites of Crustacea has accumulated (Giard and Bonnier, 1887; 1895; Smith, 1906; Shiino, 1942a,b; Veillet, 1945; Reinhard, 1944, 1956; Baer, 1951; Nicol, 1967; Hartnoll, 1967). Many rhizocephalan and epicaridean parasites may cause changes which have been termed "parasitic castration": sterilization of females and modification of male secondary sex characteristics. This phenomenon may be important in the population dynamics of some crustacean species of economic importance, as pointed out by Kuris (1974).

C. Copepods

A number of species of copepods, particularly those of the family Choniostomatidae, are parasitic on Crustacea (Hansen, 1897, 1904, 1923). One of these, *Nicothoë astaci*, known as the gill maggot, occurs on the gills of the European lobster, *Homarus gammarus*. The parasite attaches to a gill filament of the lobster by means of a suckorial mouth, pierces the filament with its mandibles, and sucks hemolymph. Newly molted lobsters are most frequently attacked, and heavily infested gills may be badly damaged by the copepods (Mason, 1959; Cressey, 1983). Because of the economic importance of their hosts, other copepods have also been reported on or in lobsters and their relatives. A harpacticoid, *Unicaleuthes*, occurs sometimes in large numbers on the exoskeleton of the American lobster, *Homarus americanus*. A copepod "related to *Anchorella*" was reported by Gordon (1966) to occur in the vas deferens of *Nephrops norvegicus*; this may be the same parasite reported earlier (but not identified) by Thomson (1896) in this location. The parasite reached a length of 4 cm and was considered to cause occlusion and degeneration of the duct. Kabata (1966, 1967) described three other copepods from *Nephrops*: *Nicothoë analata* from *Nephrops sinensis* taken in the South China Sea; *Nicothoë brucei* from *Nephrops sagamiensis* from Japan and *Nephrops andamanicus* from South Africa; and *Nicothoë simplex* from *Nephrops japonicus* from Japan.

Copepods are also known as parasites of crab eggs. Connolly (1929) described *Choniosphaera cancrorum* from the egg masses of two species of American cancrroid crabs, *Cancer borealis* and *Cancer irroratus*. Later, Gnanamuthu (1954) described *Choniosphaera indica* from the gills and egg masses of an Indian edible crab, *Neptunus sanguinolentus*. Copepod larvae were found between the crab's gill lamellae, probably feeding on tissue fluids; adults apparently suck out the fluids of the crab eggs.

IV. CONCLUSIONS

This chapter seems remarkably short, probably because the list of severe pathogens is also short. Of all of the crustacean parasites of shellfish, the rhizocephalans of crabs are probably the most significant from the viewpoint of effects on the host population. These internal parasites "inhibit growth, terminate reproduction, and remove infected individuals from the fishery" (Overstreet, 1979). Known as parasitic castrators, as well as possible causes of mortalities, the rhizocephalans have been identified as important components of population regulation systems (Kuris, 1974). The reported occurrence of periodic epizootics (Overstreet, 1983)

lends further credence to the inference of substantial damage to host populations. Additionally, inhibition of growth implies that, for species such as the blue crab, infected individuals do not reach market size. Some of the epicaridean isopods may also cause parasitic castration, and, for those that do, the host population effects would be similar to those caused by the rhizocephalans, although probably to a lesser degree.

Copepod parasites, especially a few members of the genus *Mytilicola*, tend to get most of the attention as pathogens of bivalve molluscs. Mussels and oysters, in Europe and elsewhere, are infected with these intestinal parasites, and major mortalities have been attributed to their effects during epizootics (although alternative explanations have been offered). Outbreaks of *Mytilicola* in European mussels have been reported for over half a century, and prevalences remain high in some stocks to the present time. *Mytilicola* from the Orient has been introduced with Pacific oysters imported to the North American West Coast and Europe and has invaded native oyster and mussel stocks. Principal effects of copepod parasitization seem to be poor growth and condition of the hosts, followed in some instances by mass mortalities under unfavorable environmental conditions.

Other crustacean parasites of shellfish—such as the pea crabs that inhabit the mantle cavity of bivalves or the copepods on the gills of lobsters and other decapod crustaceans—are of minor significance to their hosts, even though they may cause some localized pathology.

With the conclusion of this chapter, the systematic treatment of shellfish disease agents—beginning with the viruses and slogging through bacteria, fungi, protozoans, and helminths—comes to an end. From this point the approach will be on a subject-matter basis, after a brief look at the noninfectious diseases (neoplasms and environmentally induced abnormalities).

REFERENCES

- Adkins, G. (1972). Notes on the occurrence and distribution of the rhizocephalan parasite (*Loxothylacus texanus* Boschma) of blue crabs (*Callinectes sapidus*) in Louisiana estuaries. *Louisiana Wildl. Fish. Comm. Tech. Bull.* **12**, 13 pp.
- Allen, J. A. (1966). Notes on the relationship of the bopyrid parasite *Hemarthrus abdominalis* (Krøyer) with its hosts. *Crustaceana* **10**, 1–6.
- Andreu, B. (1960). Dispersión de *Mytilicola intestinalis* Steuer en el mejillón de cultivo a flote de la rías de Arosa y Vigo (N.W. de España). *Reun. Prod. Pesq.* **4th** pp. 115–118.
- Andreu, B. (1961). Un parásito del mejillón. Propagación del copépodo parásito *Mytilicola intestinalis* en el mejillón de la rías bajas. *Rev. Econ. Galicia, Vigo* **17–18**, 12–18.

- Andreu, B. (1963). Propagación del copépodo parásito *Mytilicola intestinalis* en el mejillón cultivado de la rías gallegas (N.W. de España). *Invest. Pesq.* **24**, 3–20.
- Andrieux, N., Herberts, C., and DeFrescheville, J. (1981). Relations hôte–parasite entre les crustacés *Carcinus* et *Sacculina carcini*: Effet d'extraits de parasite et de l'hémolymph de crabe infesté sur le protéinogramme de crabes sains. *Ann. Parasitol.* **56**, 441–448.
- Atkins, D. (1933). *Pinnotherion vermiforme* Giard and Bonnier, an entoniscid infecting *Pinnotheres pisum*. *Proc. Zool. Soc. London* **1933**, 319–363.
- Awati, P. R., and Rai, H. S. (1931). *Ostrea cucullata* (the Bombay oyster). *Indian Zool. Mem.* **3**, 1–107.
- Baer, J. G. (1951). "Ecology of Animal Parasites." University of Illinois Press, Urbana, Illinois.
- Baird, R. H., Bolster, G. C., and Cole, H. A. (1950). *Mytilicola intestinalis* Steuer in the European flat oyster (*Ostrea edulis*). *Nature (London)* **168**, 560.
- Bang, F. B. (1983). Crustacean disease responses. In "The Biology of Crustacea: Vol. 6. Pathobiology" (A. J. Provenzano, Jr. ed.), pp. 113–153. Academic Press, New York.
- Bassedas, M. (1950). Sobre la presencia de *Mytilicola intestinalis* Steuer en Barcelona. *Publ. Inst. Biol. Apl. (Barcelona)* **7**, 153–154.
- Beck, J. T. (1980). The effects of an isopod castrator, *Probopyrus pandalicola*, on the sex characters of one of its caridean shrimp hosts, *Palaemonetes paludosus*. *Biol. Bull. (Woods Hole, Mass.)* **158**, 1–15.
- Bernard, F. R. (1969). The parasitic copepod *Mytilicola orientalis* in British Columbia bivalves. *J. Fish. Res. Board Can.* **26**, 190–191.
- Berner, L. (1952). Biologie de *Pinnotheres pisum* Penn. (Décapode brachyoure). *Bull. Soc. Zool. Fr.* **77**, 344–349.
- Bishop, R. K., and Cannon, L. R. G. (1979). Morbid behavior of the commercial sand crab, *Portunus pelagicus* (L.), parasitized by *Sacculina granifera* Boschma, 1973 (Cirripedia: Rhizocephala). *J. Fish Dis.* **2**, 131–144.
- Bolster, G. C. (1954). The biology and dispersal of *Mytilicola intestinalis* Steuer, a copepod parasite of mussels. *Minist. Agric. Fish., Fish. Invest., Lond.* **18**(6), 1–30.
- Boschma, H. (1928). Rhizocephala of the North Atlantic region. *Dan. Ingolf-Exped.* **3**(10), 1–49.
- Boschma, H. (1935). Notes on Japanese Rhizocephala, with description of two new species. *Zool. Meded. Leiden* **18**, 151–160.
- Boschma, H. (1963). A rhizocephalan parasite of the crab *Charybdis callianassa* (Herbst). *Proc. K. Ned. Akad. Wet., Ser. C* **66**, 132–138.
- Boschma, H., and Haynes, E. (1969). Occurrence of the rhizocephalan *Briarosaccus callosus* Boschma in the king crab *Paralithodes camtschatica* (Tilesius) in the northeast Pacific Ocean. *Crustaceana* **16**, 97–98.
- Brian, A., and Parenzan, P. (1967). Nota sull'epicarideo *Bopyrus squillarum* Lam. parassita de Palaemonidae. *Thalassia Salentina* **2**, 37–41.
- Brienne, H. (1964). Observations sur l'infestation des moules de pertuis Breton par *Mytilicola intestinalis* Steuer. *Rev. Trav. Inst. Pêches Marit.* **28**, 205–230.
- Cantacuzène, J. (1925). Réactions de crabe sacculine vis à vis d'une infection expérimentale de la sacculine. *C. R. Soc. Biol.* **93**, 1417–1419.
- Caspers, H. (1939). Über Vorkommen und Metamorphose von *Mytilicola intestinalis* Steuer (Copepoda parasitica) in der südlichen Nordsee. *Zool. Anz.* **126**, 161–171.
- Chew, K. K., Sparks, A. K., and Katkansky, S. C. (1964). First record of *Mytilicola orientalis* Mori in the California mussel *Mytilus californianus* Conrad. *J. Fish. Res. Board Can.* **21**, 205–207.

- Chew, K. K., Sparks, A. K., and Katkansky, S. C. (1965). Preliminary results on the seasonal size distribution of *Mytilicola orientalis* and the effect of this parasite on the condition of the Pacific oyster, *Crassostrea gigas*. *J. Fish. Res. Board Can.* **22**, 1099–1101.
- Cole, H. A., and Savage, R. E. (1951). The effect of the parasitic copepod, *Mytilicola intestinalis* (Steuer) upon the condition of mussels. *Parasitology* **41**, 156–161.
- Connolly, C. J. (1929). A new copepod parasite *Choniosphaera cancrorum*, gen. et sp. n., representing a new genus, and its larval development. *Proc. Zool. Soc. London* **Part 3**, pp. 415–427.
- Cressey, R. F. (1983). Crustaceans as parasites of other organisms. In "The Biology of Crustacea: Vol. 6. Pathobiology" (A. J. Provenzano, Jr., ed.), Academic Press, New York.
- Dahl, E. (1949). Epicaridea and Rhizocephala from northern Norway with a discussion on the bathymetrical distribution of Rhizocephala. *Tromsø Mus. Aarrsh.* **69**, 1–44.
- Dare, P. J. (1982). The susceptibility of seed oysters of *Ostrea edulis* L. and *Crassostrea gigas* Thunberg to natural infestation by the copepod *Mytilicola intestinalis* Steuer. *Aquaculture* **26**, 201–211.
- Davey, J. T., and Gee, J. M. (1976). The occurrence of *Mytilicola intestinalis* Steuer, an intestinal copepod parasite of *Mytilus*, in the south-west of England. *J. Mar. Biol. Assoc. U.K.* **56**, 85–94.
- Davey, J. T., Gee, J. M., and Moore, S. L. (1978). Population dynamics of *Mytilicola intestinalis* in *Mytilus edulis* in south-west England. *Mar. Biol. (Berlin)* **45**, 319–327.
- Day, J. H. (1935). The life history of *Sacculina*. *Q. J. Microsc. Sci.* **77**, 549–583.
- Delage, M. Y. (1884). Evolution de la sacculine (*Sacculina carcini* Thomps.) crustacé endoparasite de l'ordre nouveau des kentronides. *Arch. Zool. Exp. Gen.* **2**, 417–738.
- Dethlefsen, V. (1972). Zur Parasitologie der Miesmuschel (*Mytilus edulis* L., 1758). *Ber. Dtsch. Wiss. Komm. Meeresforsch.* **22**, 344–371.
- Dethlefsen, V. (1975). The influence of *Mytilicola intestinalis* Steuer on the meat content of the mussel *Mytilus edulis* L. *Aquaculture* **6**, 83–97.
- Dinamani, P., and Gordon, D. B. (1974). On the habits and nature of association of the copepod *Pseudomyicola spinosus* with the rock oyster *Crassostrea glomerata* in New Zealand. *J. Invertebr. Pathol.* **24**, 305–310.
- Dollfus, R. P. (1914). *Trochicola enterica* nov. gen. nov. sp., eucopépode parasite de l'intestin des troques. *C. R. Hebd. Seances Acad. Sci., Ser. D* **158**, 1528–1531.
- Dollfus, R. P. (1951). Le copépode *Mytilicola intestinalis* A. Steuer peut-il être la cause d'une maladie épidémique des moules? *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **17**(2), 81–84.
- Ellenby, C. (1947). A copepod parasite of the mussel new to the British fauna. *Nature (London)* **159**, 645–646.
- Fleury, G., Lubet, P., and Ledantec, J. (1951). Note sur la *Mytilicola intestinalis* Steuer. *Ann. Pharmacol. Fr.* **9**, 569–574.
- Foxon, G. E. H. (1940). Notes on the life history of *Sacculina carcini* Thompson. *J. Mar. Biol. Assoc. U.K.* **24**, 253–264.
- Gee, J. M., Maddock, L., and Davey, J. T. (1977). The relationship between infestation by *Mytilicola intestinalis* Steuer (Copepoda, Cyclopoidea) and the condition index of *Mytilus edulis* in southwest England. *J. Cons. Perm. Int. Explor. Mer* **37**, 300–308.
- Genovese, S. (1959). Sulla presenza di *Mytilicola intestinalis* Steuer (Copepoda Parasitica) nel lago di Ganzirri. *Atti Soc. Peloritana Sci. Fis., Mat. Nat.* **5**, 47–53.
- Giard, A. (1888). La castration parasitaire, nouvelles recherches. *Bull. Sci. Fr. Belg.* **19**, 12–45.
- Giard, A., and Bonnier, J. (1887). Contributions à l'étude des bopyriens. *Trav. Stn. Zool. Wimereux* **5**, 1–252.
- Giard, A., and Bonnier, J. (1895). Contributions à l'étude des épicarides. XX. Sur les

- épicarides parasites des arthrostaces et sur quelques copépods symbiotes de cas épicarides. *Bull. Sci. Fr. Belg.* **25**, 417–493.
- Gnanamuthu, C. P. (1954). *Choniosphaera indica*, a copepod parasitic on the crab *Neptunus* sp. *Parasitology* **44**, 371–378.
- Gordon, I. (1966). Parasites and diseases of Crustacea. *Mem. Inst. Fond. Afr. Noire* **77**, 27–86.
- Grainger, J. N. R. (1951). Notes on the biology of the copepod *Mytilicola intestinalis* Steuer. *Parasitology* **41**, 135–142.
- Hansen, H. J. (1897). "The Choniostomatidae. A Family of Copepoda, Parasites on Crustacea Malacostraca." Høst, Copenhagen.
- Hansen, H. J. (1904). Two new forms of Choniostomatidae: Copepoda parasitic on Crustacea Malacostraca and Ostrocods. *Q. J. Microsc. Sci.* **48**, 347–358.
- Hansen, H. J. (1923). Crustacea Copepoda. II. Copepoda parasita and hemiparasita. *Dan. Ingolf-Expd.* **3**, 1–92.
- Hartnoll, R. G. (1967). The effects of sacculinid parasites on two Jamaican crabs. *J. Linn. Soc. Lond., Zool.* **46**, 275–295.
- Haven, D. (1959). Effects of pea crabs *Pinnotheres ostreum* on oysters *Crassostrea virginica*. *Proc. Natl. Shellfish. Assoc.* **49**, 77–86.
- Havinga, B. (1951). *Mytilicola intestinalis* in relation to other invasions of aquatic animals. *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **17**, 77–80.
- Hawkes, C. R., Meyers, T. R., and Shirley, T. C. (1985). Parasitism of the blue king crab, *Paralithodes platypus*, by the rhizocephalan, *Briarosaccus callosus*. *J. Invertebr. Pathol.* **45**, 252–253.
- Heldt, J. H. (1951). Observations sur *Mytilicola intestinalis* Steuer parasite des moules. *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **17**, 33–40.
- Hepper, B. T. (1953). Artificial infection of various molluscs with *Mytilicola intestinalis* Steuer. *Nature (London)* **172**, 250.
- Hepper, B. T. (1955). Environmental factors governing the infection of mussels *Mytilus edulis* by *Mytilicola intestinalis*. *Minist. Agric. Fish. Food, Fish. Invest., Lond.* **20**, 1–21.
- Herberts, C. (1982). Host–parasite relation between the shore crab *Carcinus maenas* and *Sacculina carcini* (Rhizocephala): Identification and characterization of a specific fraction correlated with parasitism. *J. Invertebr. Pathol.* **39**, 60–65.
- Hiraiwa, Y. K., and Sato, M. (1939). On the effect of parasitic Isopoda on a prawn, *Penaeopsis akayebi* Rathbun, with a consideration of the effect of parasitization on the higher Crustacea in general. *J. Sci. Hiroshima Univer., Ser. B* **7** (Article 6), 105–124.
- His, É. (1977). Observations préliminaires sur la présence de *Mytilicola orientalis* Mori (1935) chez *Crassostrea gigas* Thunberg dans le Bassin d'Arcachon. *Bull. Soc. Geol. Amis Mus. Havre* **64**, 7–8.
- His, É. (1979). Mytilicolides et mycolides parasites des lamellibranches d'intérêt commercial du bassin d'Arcachon. *Haliotis* **8**, 99–102.
- Hockley, A. R. (1952). On the biology of *Mytilicola intestinalis* (Steuer). *J. Mar. Biol. Assoc. U.K.* **30**, 223–232.
- Hopkins, S. H. (1957). Interrelations of organisms. B. Parasitism. *Mem. Geol. Soc. Am.* **67**, 413–428.
- Horsted, S. A., and Smidt, E. (1956). The deep sea prawn (*Pandalus borealis* Kr.) in Greenland waters. *Medd. Komm. Havunders., Kjobenhaven (New Ser.)* **1**(11), 1–118.
- Hoshina, T., and Kuwabara, R. (1959). On a new commensal copepod, *Mytilicola mactrae* n. sp. obtained from the intestine of *Mactra veneriformis* Reeve. *J. Tokyo Univ. Fish.* **45**, 33–35.
- Hrs-Brenko, M. (1964). *Mytilicola intestinalis* Steuer as a parasite of mussels in natural beds and artificial rearing places of the eastern Adriatic. *Acta Adriat.* **11**, 161–165.

- Hubert, M., Chassard-Bouchaud, C., and Bocquet-Védrine, J. (1976). Cytologie—aspects ultrastructuraux des hémocytes de *Carcinus maenas* L. (Crustacé Décapode), parasité par *Sacculina carcini* Thompson (Crustacé Cirripède): Activité réactionnelle, genèse de collagène. *C. R. Hebd. Seances Acad. Sci., Ser. D* **283**, 789–792.
- Humes, A. G. (1954). *Mytilicola porrecta* n. sp. (Copepoda: Cyclopoida) from the intestine of marine pelecypods. *J. Parasitol.* **40**, 186–194.
- Ichikawa, A., and Yamagunachi, R. (1957). The sexual nature of a rhizocephalan, *Pelto-gasterella socialis*. *J. Fac. Sci., Hokkaido Univ. Ser. 6* **13**, 384–389.
- Johnson, P. T., MacIntosh, R. A., and Somerton, D. A. (1986). Rhizocephalan infection in blue king crabs, *Paralithodes platypus*, from Olga Bay, Kodiak Island, Alaska. *Fish. Bull.* **84**, 177–184.
- Kabata, Z. (1966). *Nicotohœ analata* sp. nov., a parasitic copepod from the south China Sea. *Crustaceana* **11**, 10–16.
- Kabata, Z. (1967). *Nicotohœ* Audouin & H. Milne-Edwards, 1826 (Crustacea, Copepoda), a genus parasitic on *Nephrops* Leach, 1816 (Crustacea, Decapoda). *Zool. Meded.* **42**, 147–161.
- Katkansky, S. C., and Warner, R. W. (1968). On the unusual occurrence of the copepod *Mytilicola orientalis* in the digestive diverticulae of the Pacific oyster, *Crassostrea gigas*. *J. Invertebr. Pathol.* **12**, 475.
- Katkansky, S. C., Sparks, A. K., and Chew, K. K. (1967). Distribution and effects of the endoparasitic copepod, *Mytilicola orientalis*, on the Pacific oyster, *Crassostrea gigas*, on the Pacific coast. *Proc. Natl. Shellfish. Assoc.* **57**, 50–58.
- Korringa, P. (1950). De aanval van de parasiet *Mytilicola intestinalis* op de Zeeuwse mosselcultuur. *Visserijnieuws* **3**, 1–7 (translated by S. H. Hopkins, Texas A&M Univ. Res. Found., 1951, mimeo).
- Korringa, P. (1951a). Over *Mytilicola intestinalis* (Copepoda parasitica) en enkele andere ongewenste vreemdelingen in onze wateren. *Vakbl. Biol.* **31**, 63–74.
- Korringa, P. (1951b). Le *Mytilicola intestinalis* Steuer (Copepoda parasitica) menace l'industrie moulière en Zelande. *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **17**, 9–13.
- Korringa, P. (1952a). Recent advances in oyster biology. *Q. Rev. Biol.* **27**, 339–365.
- Korringa, P. (1952b). Epidemiological observations on the mussel parasite *Mytilicola intestinalis* Steuer, carried out in The Netherlands 1951. *Ann. Biol.* **8**, 182–185.
- Korringa, P. (1953). Epidemiological observations on the mussel parasite *Mytilicola intestinalis* Steuer carried out in the Netherlands 1952. *Ann. Biol.* **9**, 219–224.
- Korringa, P. (1957). Epidemiological observations on the mussel parasite *Mytilicola intestinalis* Steuer, undertaken in The Netherlands in 1955. *Ann. Biol.* **12**, 230–231.
- Korringa, P. (1959). Checking *Mytilicola's* advance in the Dutch Waddensea. *Meet. Cons. Perm. Int. Explor. Mer, 45th: Shellfish Comm. Rep.* **87**, 1–3.
- Korringa, P. (1968). On the ecology and distribution of the parasitic copepod *Mytilicola intestinalis* Steuer. *Bijdr. Dierk.* **38**, 47–57.
- Korringa, P., and Lambert, L. (1951). Quelques observations sur la fréquence de *Mytilicola intestinalis* Steuer (Copepoda parasitica) dans les moules du littoral méditerranéen français avec une note sur la présence de *Pseudomyicola spinosus* (Raff. & Mont.) (Copepoda parasitica). *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **17**, 15–29.
- Kuris, A. M. (1974). Trophic interactions: Similarity of parasitic castrators to parasitoids. *Q. Rev. Biol.* **49**, 129–148.
- Kuris, A. M., Poinar, G. O., and Hess, R. T. (1980). Post-larval mortality of the endoparasitic isopod castrator *Portunium conformis* (Epicaridea: Entoniscidae) in the shore crab, *Hemigrapsus oregonensis*, with a description of the host response. *Parasitology* **80**, 211–232.

- Lambert, L. (1951a). Renseignements pouvant aider à la reconnaissance de *Mytilicola* et de ses larves et description des méthodes d'examen d'échantillons de moules. *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **17**, 41–46.
- Lambert, L. (1951b). Le cop rouge (*Mytilicola intestinalis* Steuer) sur les côtes de France. *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **17**, 51–56.
- Lauckner, G. (1983). Diseases of Mollusca: Bivalvia. In "Diseases of Marine Animals" (O. Kinne, ed.), Vol. II, pp. 477–961. Biol. Anstalt Helgol., Hamburg, FRG.
- Lawler, A. R., and Shepard, S. L. (1979). A bibliography of the Rhizocephala (Crustacea: Cirripedia). *Gulf Res. Rep.* **6**, 153–167.
- Leloup, E. (1951). Sur la présence de *Mytilicola intestinalis* Steuer de long de la côte de Belgique. *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **17**, 57–58.
- Leloup, E. (1960). Recherches sur la répartition de *Mytilicola intestinalis* Steuer, 1905, le long de la côte Belge (1950–1958). *Int. R. Sci. Nat. Belg., Bull.* **36**, 1–12.
- Lester, R. J. G. (1978). Marine parasites costly for fishermen. *Aust. Fish.* **37**, 32–33.
- Mann, H. (1951). Qualitätsverminderung der Fleisches der Miesmuscheln durch den Befall mit *Mytilicola intestinalis*. *Fischereiwelt* **3**, 121–122.
- Mann, H. (1956). The influence of *Mytilicola intestinalis* (Copepoda parasitica) on the development of the gonads of *Mytilus edulis*. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **140**, 57–58.
- Mason, J. (1959). The biology of *Nicthoë astaci* Audoin and Milne-Edwards. *J. Mar. Biol. Assoc. U.K.* **38**, 3–16.
- Matsumoto, K. (1953). On the epicaridization of the fresh-water crab *Eriocheir japonicus*. *Dobutsugaku Zasshi* **62**, 354–361 (in Japanese; English summary).
- McDermott, J. J. (1962). The incidence and host-parasite relations of pinnotherid crabs (Decapoda, Pinnotheridae). *Proc. Natl. Coastal Shallow Water Res. Conf., 1st NSF-ONR*, 162–164.
- McMullen, J. C., and Yoshihara, H. T. (1970). An incidence of parasitism of deepwater king crab, *Lithodes aquispina*, by the barnacle *Briarosaccus callosus*. *J. Fish. Res. Board Can.* **27**, 818–821.
- Meyer, P. F., and Mann, H. (1950). Beiträge zur Epidemiologie und Physiologie des parasitischen Copepoden *Mytilicola intestinalis*. *Arch. Fischereiwiss.* **2**, 120–134.
- Meyer, P. F., and Mann, H. (1951). Recherches allemandes relatives au "Mytilicola," copépode parasite de la moule, existant dans les watten allemandes 1950/51. *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **17**, 63–74.
- Meyer, P. F., and Mann, H. (1952a). *Mytilicola*—Epidémie 1951–52. *Fischereiwelt* **4**, 136.
- Meyer, P. F., and Mann, H. (1952b). Ein weiterer Beitrag zur Epidemiologie von *Mytilicola intestinalis*. *Arch. Fischereiwiss.* **5**, 26–34.
- Meyer-Waarden, P. F., and Mann, H. (1954a). Der Befall von *Mytilus edulis* durch *Mytilicola intestinalis* in den deutschen Wattengebieten 1950–53. *Ber. Dtsch. Komm. Meeresforsch.* **13**, 347–362.
- Meyer-Waarden, P. F., and Mann, H. (1954b). Untersuchungen über die Bestände von *Mytilus galloprovincialis* an der italienischen Küste auf ihren Befall mit *Mytilicola intestinalis* (Copepoda parasitica). *Boll. Pesca, Piscic. Idrobiol. (New Ser.)* **8**, 5–24.
- Meyer-Waarden, P. F., and Mann, H. (1956). German investigations with respect to *Mytilicola intestinalis* in *Mytilus edulis* in 1953. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **140**, 54–56.
- Møller-Christensen, A., and McDermott, J. J. (1958). Life-history and biology of the oyster crab, *Pinnotheres ostreum* Say. *Biol. Bull. (Woods Hole, Mass.)* **114**, 146–179.
- Monod, T., and Dollfus, R. P. (1932). Les copépodes parasites de mollusques. *Ann. Parasitol. Hum. Comp.* **10**, 129–204.

- Mori, T. (1935). *Mytilicola orientalis*, a new species of parasitic Copepoda *Dobutsugaku Zasshi* **47**, 687–690. (in Japanese; English summary).
- Nicol, J. A. C. (1967). "The Biology of Marine Animals." Pitman, New York.
- Odlaug, T. O. (1946). The effect of the copepod, *Mytilicola orientalis*, upon the Olympia oyster, *Ostrea lurida*. *Trans. Am. Microsc. Soc.* **65**, 311–317.
- Overstreet, R. M. (1978). Marine maladies? Worms, germs, and other symbionts from the northern Gulf of Mexico. *Miss.-Ala. Sea Grant Consortium MASGP-78-021*, 140 pp.
- Overstreet, R. M. (1979). Metazoan symbionts of the blue crab. *Proc. Blue Crab Colloq.* pp. 81–87.
- Overstreet, R. M. (1983). Metazoan symbionts of crustaceans. In "The Biology of Crustacea: Vol. 6. Pathobiology" (A. J. Provenzano, Jr. ed.), pp. 155–250. Academic Press, New York.
- Paul, J. D. (1983). The incidence and effects of *Mytilicola intestinalis* in *Mytilus edulis* from the rias of Galicia, northwest Spain. *Aquaculture* **31**, 1–10.
- Pearce, J. B. (1966). The biology of the mussel crab, *Fabia subquadrata*, from the waters of the San Juan Archipelago, Washington. *Pac. Sci.* **20**, 3–35.
- Pérez, C. (1903). Sur un isopode parasite d'une sacculine. *Proc. Verb. Seances Soc. Sci. Phys. Nat. Bordeaux* pp. 109–110.
- Pérez, C. (1927). Notes sur les épicarides et les rhizocéphales des côtes de France. I. Sur l'"*Eupagurus bernhardus*" et sur quelques-uns de ses parasites. *Bull. Soc. Zool. Fr.* **52**, 99–104.
- Pérez, C. (1928). Notes sur les épicarides et les rhizocéphales des côtes de France. II. Nouvelles observations sur les parasites de l'"*Eupagurus bernhardus*". III. L'"*Eupagurus cuanensis*" et ses parasites. *Bull. Soc. Zool. Fr.* **53**, 523–526.
- Pérez, C. (1929). Notes sur les épicarides et les rhizocéphales des côtes de France. V. Non-spécificité du parasitisme du *Liriopsis pygmaea*. *Bull. Soc. Zool. Fr.* **54**, 607–609.
- Pérez, C. (1931a). Notes sur les épicarides et les rhizocéphales des côtes de France. VII. *Peltogaster* et *Liriopsis*. *Bull. Soc. Zool. Fr.* **56**, 509–512.
- Pérez, C. (1931b). Statistique d'infestation des pagures par les *Chlorogaster*. *C. R. Acad. Sci.* **192**, 1274–1276.
- Pérez, C. (1931c). Les rhizocéphales parasites des pagures. *Verh. Schweiz. Naturforsch. Ges.* **112**, 261–276.
- Pesta, O. (1907). Die Metamorphose von *Mytilicola intestinalis* Steuer. *Z. Wiss. Zool.* **88**, 77–98.
- Phillips, W. J., and Cannon, L. R. G. (1978). Ecological observations on the commercial sand crab, *Portunus pelagicus* (L.), and its parasite, *Sacculina granifera* Boschma, 1973 (Cirripedia: Rhizocephala). *J. Fish Dis.* **1**, 137–149.
- Poisson, H. (1946). Huitres et ostréiculture à Madagascar. Étude zoologique et économique. *Trav. Sect. Oceanogr. Appl. Soc. Amis Parc Botan. Zool. Tananariva* **3**, 1–37.
- Potts, F. A. (1906). The modification of the sexual characters of the hermit crab caused by the parasite *Peltogaster* (castration parasitaire of Giard). *Q. J. Microsc. Sci.* **50**, 599–621.
- Potts, F. A. (1915). On the rhizocephalan genus *Thompsonia* and its relation to the evolution of the group. *Carnegie Inst. Washington, Pap. Dep. Mar. Biol.* **8**, 1–32.
- Pregenzer, C. (1978). *Pinnotheres hickmani* (Guiler) in wild and cultured *Mytilus edulis* in Port Phillip Bay, Victoria. *Aust. J. Mar. Freshwater Res.* **29**, 127–139.
- Reinhard, E. G. (1942). Studies on the life history and host-parasite relationship of *Peltogaster paguri*. *Biol. Bull. (Woods Hole, Mass.)* **83**, 401–415.
- Reinhard, E. G. (1944). Rhizocephalan parasites of hermit crabs from the Northwest Pacific. *J. Wash. Acad. Sci.* **34**, 49–58.

- Reinhard, E. G. (1945). *Paguritherium alatum* n. g. n. sp., an entoniscan parasite of *Pagurus longicarpus*. *J. Parasitol.* **31**, 198–204.
- Reinhard, E. G. (1950). An analysis of the effects of a sacculinid parasite on the external morphology of *Callinectes sapidus* Rathbun. *Biol. Bull. (Woods Hole, Mass.)* **98**, 277–288.
- Reinhard, E. G. (1956). Parasitic castration of Crustacea. *Exp. Parasitol.* **5**, 79–107.
- Reinhard, E. G., and Buckeridge, F. W. (1950). The effect of parasitism by an entoniscid on the secondary sex characters of *Pagurus longicarpus*. *J. Parasitol.* **36**, 131–138.
- Reverberi, G. (1943). Le castrazione parassitaria e la determinazione del sesso nei crostacei. *Attual. Zool.* **1943**, 1.
- Reverberi, G. (1952). Parasitismo, iperparassitismo e sesso nei crostacei. *Pubbl. Stn. Zool. Napoli* **23**, 285–296.
- Ricketts, E. F., and Calvin, J. (1962). "Between Pacific Tides," 3rd rev. ed. Stanford University Press, Stanford, California.
- Rubiliani, C. (1985). Response of two species of crabs to a rhizocephalan extract. *J. Invertebr. Pathol.* **45**, 304–310.
- Sandoz, M., and Hopkins, S. H. (1947). Early life history of the oyster crab, *Pinnotheres ostreum* (Say). *Biol. Bull. (Woods Hole, Mass.)* **93**, 250–258.
- Seed, R. (1969). The incidence of the pea crab, *Pinnotheres pisum*, in the two types of *Mytilus* (Mollusca: Bivalvia) from Padstow, south-west England. *J. Zool.* **158**, 413–420.
- Shiino, S. M. (1931). Studies in the modification of sexual characters in *Eupagurus samuelis* caused by a rhizocephalan parasite *Peltogaster* sp. *Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B* **7**, 63–101.
- Shiino, S. M. (1933). Bopyrids from Tanabe Bay. I. *Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B* **8**, 249–300.
- Shiino, S. M. (1934). Bopyrids from Tanabe Bay. II. *Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B* **9**, 258–287.
- Shiino, S. M. (1936). Bopyrids from Tanabe Bay. III. *Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B* **11**, 157–174.
- Shiino, S. M. (1937). Bopyrids from Tanabe Bay. IV. *Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B* **12**, 479–493.
- Shiino, S. M. (1939a). Bopyrids from Tanabe Bay. V. *Annot. Zool. Jpn.* **18**, 11–16.
- Shiino, S. M. (1939b). Bopyrids from Kyushyu and Ryukyu. *Rec. Oceanogr. Works Jpn.* **10**, 79–99.
- Shiino, S. M. (1942a). Bopyrids from the South Sea Islands with description of a hyperparasitic cryptoniscid. *Palao Trop. Biol. Stud.* **2**, 437–458.
- Shiino, S. M. (1942b). On the parasitic isopods of the family Entoniscidae, especially those found in the vicinity of Seto. *Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B* **17**, 37–76.
- Shiino, S. M. (1943). Rhizocephala of Japan. *J. Sigenkagku Kenkyusyo* **1**, 1–36.
- Shiino, S. M. (1949). On two new species of the bopyrid genus *Bopyrella* found in Japan. *Bull. Biogeograph. Soc. Jpn.* **14**, 45–50.
- Shiino, S. M. (1958). Note on the bopyrid fauna of Japan. *Rep. Fac. Fish., Prefect. Univ. Mie* **3**, 29–73.
- Shiino, S. M. (1964a). On three bopyrid isopods from California. *Rep. Fac. Fish., Prefect Univ. Mie* **5**, 19–25.
- Shiino, S. M. (1964b). On two species of bopyrid isopods parasitic on *Callianassa uncinata* Milne-Edwards from Chile. *Rep. Fac. Fish., Prefect. Univ. Mie* **5**, 27–32.
- Shiino, S. M. (1964c). Results of Amami expedition. 5. Bopyridae. *Rep. Fac. Fish., Prefect. Univ. Mie* **5**, 237–242.
- Shiino, S. M. (1965). Phylogeny of the genera within the family Bopyridae. *Bull. Mus. Nat. Hist. Nat.* **37**, 462–465.

- Smith, G. W. (1906). Rhizocephala. *Fauna Flora Neapel* **29**, 1–123.
- Sparks, A. K. (1962). Metaplasia of the gut of the oyster *Crassostrea gigas* (Thunberg) caused by infection with the copepod *Mytilicola orientalis* Mori. *J. Insect Pathol.* **4**, 57–62.
- Sparks, A. K. (1985). "Synopsis of Invertebrate Pathology Exclusive of Insects," 423 pp. Elsevier, Amsterdam.
- Stauber, L. A. (1945). *Pinnotheres ostreum*, parasite of the American oyster, *Ostrea* (*Gryphaea*) *virginica*. *Biol. Bull. (Woods Hole, Mass.)* **88**, 269–291.
- Steuer, A. (1902). *Mytilicola intestinalis* n. gen. n. sp. aus dem Darne von *Mytilus galloprovincialis* Lam. *Zool. Anz.* **25**, 635–637.
- Steuer, A. (1903). *Mytilicola intestinalis* n. gen. n. sp. *Arb. Zool. Inst. Univ. Wien* **15**, 1–45.
- Thomas, H. J. (1953). *Mytilicola intestinalis* Steuer in England and Wales. Progress report 1953. *Cons. Perm. Int. Explor. Mer, Rep. Shellfish. Comm.* pp. 1–2.
- Thomson, J. S. (1896). A copepod parasite of *Nephrops norvegicus*. *Proc. R. Soc. Edinburgh* **13**, 246–250.
- Tucker, B. W. (1930). On the effects of an epicaridian parasite, *Gyge branchialis* on *Upogebia littoralis*. *Q. J. Microsc. Sci.* **74**, 1–118.
- Van Arman, J. A., and Smith, A. C. (1970). The pathobiology of an epibranchial bopyrid isopod in a shrimp, *Hippolytina wurdemanni*. *J. Invertebr. Pathol.* **15**, 133–135.
- Veillet, A. (1945). Recherches sur le parasitisme des crabes et des galathées par les rhizocephales et les épicarides. *Ann. Inst. Oceanogr. (Monaco)* **22**, 193–341.
- Waterman, T. H. (Ed.) (1960). "The Physiology of Crustacea," Vol. 1. Academic Press, New York.
- Waterman, T. H. (Ed.) (1961). "The Physiology of Crustacea," Vol. 2. Academic Press, New York.
- Waugh, G. D. (1954). The occurrence of *Mytilicola intestinalis* (Steuer) on the east coast of England. *J. Anim. Ecol.* **23**, 364–367.
- Waugh, G. D. (1966). Protecting British shellfisheries. *Minist. Agric. Fish. Food, Fish. Lab., Burnham on Crouch, Leaflet. (New Ser.)* **10**, 1–8.
- Williams, A. B. (1965). Marine decapod crustaceans of the Carolinas. *U.S. Fish Wildl. Serv., Fish. Bull.* **65**, 1–298.
- Williams, C. S. (1969). The life history of *Mytilicola intestinalis* Steuer. *J. Cons. Perm. Explor. Mer* **32**, 419–429.
- Wilson, C. B. (1938). A new copepod from Japanese oysters transplanted to the Pacific coast of the United States. *J. Wash. Acad. Sci.* **28**, 284–288.
- Yamaguti, S. (1939). A new commensal copepod from *Brachidontes senhousi* (Reeve). *Trans. Am. Microsc. Soc.* **58**, 371–373.
- Yoshida, M. (1952). On the breeding character of the shrimp, *Leander serrifer*, parasitized by bopyrids. *Annot. Zool. Jpn.* **25**, 362–365.

Part III

Noninfectious Diseases of Shellfish

This section of Volume 2 represents an attempt to treat two reasonably disparate subjects—neoplastic diseases (Chapter 8) and genetic and environmentally induced diseases (Chapter 9)—under the general heading “*Noninfectious Diseases*.” Quickly perceived problems here are the possible viral (hence, infectious) etiology of some of the shellfish neoplasias and the complex (and largely undetermined) influences of genetics, nutrition, and environment in producing some of the abnormalities seen.

There are legitimate reasons for giving some attention to these noninfectious diseases, despite the predominant role of the infectious diseases—outlined in the first seven chapters—in affecting survival of shellfish. These include the following:

- Some of the neoplastic conditions in bivalve molluscs can be lethal and can occur at epizootic levels, thereby demanding serious consideration in any discussion of disease effects on populations.
- Sublethal morphological abnormalities seen in juvenile and adult animals may be only thin shadows of much larger lethal effects of defective heredity and submarginal environments on larvae and postlarvae—effects which extract abnormal individuals from the population at their earliest life history stages, long before they are recruited to the fishery.
- The appearance of morphological abnormalities in aquaculture populations (such as blunted heads and cramped tails in shrimp) may signal the presence of marginal nutritional or environmental conditions, which, if uncorrected, may lead to mortalities or the onset of infectious disease outbreaks. Also, these morphological anomalies may mirror counterpart physiological disorders, which could result in poor growth, reproductive impairment, or death.

- The question of possible relationships of coastal/estuarine pollution and abnormalities in shellfish is a persistent one; there are some indications that such a relationship may exist (e.g., excretory concretions in bivalves from polluted habitats or malformed oyster shells resulting from exposure to tributyl tin); but there are other indications that a relationship may not exist (e.g., hematopoietic neoplasms in clams and petroleum contamination).

For these reasons—and to preserve a façade of balanced treatment of infectious and noninfectious processes—the neoplastic conditions (especially in bivalve molluscs) and the various morphological anomalies in molluscs and crustaceans are accorded two chapters. It should be recognized, though, that, except for the hematopoietic neoplasias of bivalve molluscs and possibly the abnormalities in larval development, the noninfectious diseases are interesting scientifically, but probably do not have a major influence on host population abundance.

8

Neoplastic Diseases

I. INTRODUCTION

This discussion of neoplasia in marine molluscs and crustaceans is remarkably unbalanced, since much of the published information about diseases of significance concerns malignant disorders of bivalve molluscs—hence, much of the text here has a similar weighting. Before moving ahead, though, into a consideration of neoplasia in economic marine shellfish, it might be good to discuss a few principles and some of the terminology involved.

A tumor may be defined broadly as “any swelling or abnormal mass of tissue” resulting from (1) nonneoplastic controlled cell proliferation (hyperplasia), (2) a nonneoplastic increase in cell size (hypertrophy), or (3) uncontrolled cell proliferation (neoplasia).

In current usage, though, the term “tumor” has become almost synonymous with “neoplasm,” despite its original, broader definition (Steinhaus and Martignoni, 1967; Lauckner, 1983).

Neoplasms (or neoplasias) may be further defined as “growth disturbances characterized by excessive, abnormal, proliferation of cells, independent of normal growth regulating mechanisms of the animal, and persisting after termination of the stimulus that initiated growth” (Sparks, 1985), or as “that form of hyperplasia which is caused, at least in part, by an intrinsic heritable abnormality in the involved cells” (Prehn, 1975).

Tumors of vertebrates have been classified in a number of ways, but one of the most useful separates “benign” from “malignant” growths, as seen in Table 10.

It should be noted though that “benign” and “malignant” are clinical terms developed for neoplasia in vertebrates; information from studies of invertebrate neoplasia is still inadequate, particularly in the demonstration of lethality, although some evidence is accumulating that

Table 10. Classification of Tumors^a

Benign (Nonfatal)	Malignant (Fatal)
Structure is typical of the tissue of origin	Structure is atypical, with imperfect differentiation (often with pleomorphic, undifferentiated cells)
Growth is slow, expansive, encapsulated	Growth is invasive, rapid, unencapsulated
Mitotic figures are infrequent and normal	Mitotic figures are abundant and abnormal
Growth may cease or regress	Growth is progressive, terminating in death
Metastasis does not occur	Metastasis occurs frequently

^a After Sparks (1985).

demonstrates a progression toward death. Until recently, most tumors of invertebrates were thought to be benign, but a number of studies of bivalve molluscs during the past two decades have disclosed carcinomas, germinomas, and presumed hemocytic sarcomas that meet the criteria of malignancy used in vertebrates and are considered to be malignant (Sparks, 1985; Farley *et al.*, 1986). Sparks (1985) noted a marked tendency by authors reporting their results of research on neoplasia in invertebrates to use vertebrate tumor terminology and to extrapolate from knowledge of vertebrate neoplasia to abnormalities in invertebrates, even when comparative relationships were not understood—despite early cautions favoring conservatism (Scharrer and Lochhead, 1950; Dawe, 1968; Sparks, 1972). Such conservatism seems well founded, since studies of neoplasia in invertebrates are subject to a number of uncertainties:

- The distinction between neoplasia and hyperplasia or response to injury or parasitism is often difficult to determine (Lauckner, 1983).
- There is some indication that certain hyperplasias may progress into neoplastic conditions, but “hard” information is scarce.
- The etiology of most invertebrate neoplasms is unknown, except for a possible viral participant in the development of hemocytic neoplasia in soft-shell clams (*Mya arenaria*). (It should be pointed out that the etiologies of most vertebrate neoplasms are also unknown.)
- The progenitor cells of the tumor may be difficult to determine.

For those interested in greater detail than is provided in this chapter, reviews of neoplasia in shellfish (and other invertebrates) have been published sporadically. The most recent have been those by Sparks

(1985) and by Mix (1986). Earlier reviews include those by Scharrer and Lochhead (1950), Dawe (1968), and Pauley (1969) (for bivalve molluscs).

II. NEOPLASTIC DISEASES OF MOLLUSCS

Having tried, in the Introduction, to distinguish malignant from benign tumors, it would seem logical to divide the discussion of neoplasms in molluscs into those two major categories, and, since this is a book about diseases with the greatest impact on fish and shellfish species, to concentrate on those disorders which have such impact: the malignant neoplasias. The dominant criterion of malignancy is that it leads to death; this criterion has been satisfied in some of the sarcomas and one carcinoma of bivalves, which have been shown to produce mortalities.

The literature on neoplasia in molluscs has been dominated since 1969 by reports of presumed hemocytic (and in a few cases gonadal) neoplasms, sometimes at epizootic levels, in oysters, mussels, clams, and cockles (Table 11). Many reports refer to the hemocytic disease as "hematopoietic neoplasia" (Yevich and Barszcz, 1977; Reinisch *et al.*, 1983). Balouet and Poder (1979), for example, suggested, but did not demonstrate, that the neoplastic cells arose from hyaline hemocytes. A more conservative and nonspecific designation of the disease as a "sarcoma" or a "sarcomatoid proliferative disease" has been adopted by Farley and associates in the absence of definitive evidence about progenitor cells (and the failure of some monoclonal antibodies developed against neoplastic cells to cross-react with normal hemocytes) (Fig. 34). Other authors (Balouet *et al.*, 1982; Poder and Auffret, 1986) have adopted a somewhat more specific but still conservative terminology, describing the disease condition as "a sarcoma, probably of hemocytic origin or corresponding to an undifferentiated process arising from hematopoietic stem cells."

The neoplastic condition is clearly progressive, highly invasive, and lethal. It has been associated with mortalities of oysters, mussels, and clams, and—at least in the case of soft-shell clams—seems to be of transmissible and possibly infectious etiology, based on field observations and experimental studies (Brown *et al.*, 1977; Farley *et al.*, 1986; Elston *et al.*, 1987).

Because the sarcomatoid proliferative disease (i.e., hemocytic sarcoma) occurs in a number of economically important bivalves and because epizootic levels may affect population abundance, it seems relevant to discuss the condition in some detail, as it occurs in each major bivalve group. Authors have used a variety of terms to describe the

Table 11. Malignant Neoplastic Diseases of Bivalve Molluscs^a

Type	Species Affected	Location	Prevalences Reported	References
Hemocytic sarcoma	<i>Crassostrea virginica</i> (American oyster)	Atlantic and Gulf coasts of the United States	0.2% (Chesapeake Bay)	Farley (1969a), Farley and Sparks (1970), Newman (1972), Frier- man (1976), Frierman and An- drews (1976), Otto and Farley (1976), Couch and Winstead (1979), Harshbarger <i>et al.</i> (1979) Farley and Sparks (1970)
Hemocytic sarcoma ^b	<i>Crassostrea gigas</i> (Pacific oyster)	Pacific coast of the United States	—	
Hemocytic sarcoma ^b	<i>Ostrea edulis</i> (Euro- pean flat oyster)	France, Spain, Yugoslavia	0.57% (France)	Franc (1975), Balouet and Poder (1978), Alderman <i>et al.</i> (1977), Balouet <i>et al.</i> (1983, 1986)
Hemocytic sarcoma	<i>Ostrea lurida</i> Olym- pia oyster)	Pacific coast of the United States	12–40%	Jones and Sparks (1969), Farley and Sparks (1970), Mix (1975a, b, 1976, 1982, 1983) Mix <i>et al.</i> (1977a)
Hemocytic sarcoma ^b	<i>Mytilus edulis</i> (blue mussel)	Pacific coast of the United States and Canada, United Kingdom	1–4.3% (England); 12.7% (Canada), with marked seasonal and geographic variability	Farley and Sparks (1970), Mix <i>et</i> <i>al.</i> (1977b, 1979a, b), Lowe and Moore (1978), Mix (1982, 1983), Green and Alderman (1983), Cosson-Mannevy <i>et al.</i> (1984)

Germinoma ^b and hemocytic sarcoma	<i>Mya arenaria</i> (soft-shell clam)	Atlantic coast of the United States	1–22% (gonadal), 42–65% (Chesapeake Bay in 1984)	Barry and Yevich (1975), Brown <i>et al.</i> (1976, 1977, 1979), Farley (1976a), Yevich and Barszcz (1976, 1977), Brousseau (1987)
Carcinoma	<i>Macoma balthica</i> (duck clam)	Atlantic coast of the United States	12–25%	Christensen <i>et al.</i> (1974), Farley (1976a,b, 1977)
Hemocytic sarcoma? ^b	<i>Macoma nasuta</i> , <i>Macoma irus</i>	Pacific coast of the United States	—	Mix <i>et al.</i> (1977b)
Hemocytic sarcoma ^b	<i>Cerastoderma edule</i> (cockle)	France, Ireland	France, 0–46% (average, 4%), with marked geographic variability; Ireland, 40% (specimens collected from one site over an 8-month period)	Poder <i>et al.</i> (1983), Auffret and Poder (1986), Poder and Auffret (1986), Twomey and Mulcahy (1984, 1988)
Gonadal neoplasm (germinoma)	<i>Mercenaria mercenaria</i>	Narragansett Bay, Rhode Island	—	Barry and Yevich (1972)
Gonadal neoplasm (germinoma) ^b	<i>Mytilus edulis</i>	British Columbia, Canada	One of 332 specimens	Cosson-Mannevy <i>et al.</i> (1984)

^a After Lauckner (1983).

^b Malignancy has been implied but not proven in some instances.

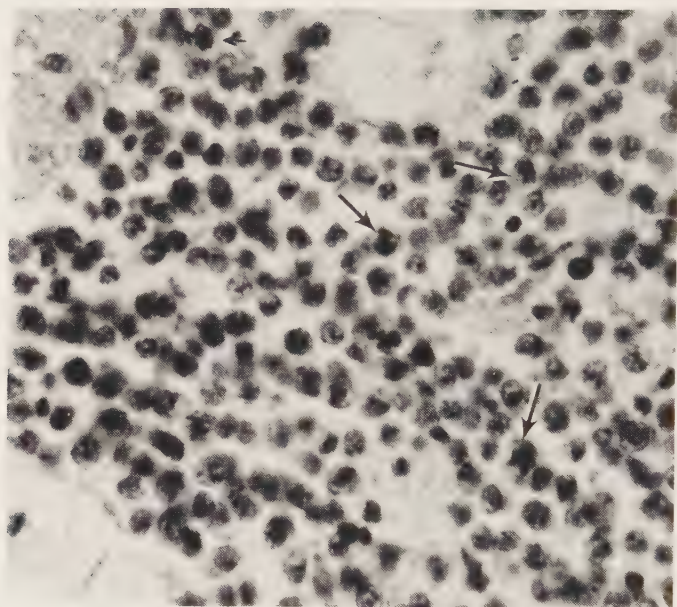


Figure 34. Molluscan Sarcomatoid Proliferative Disease. Note enlarged nuclei and numerous mitotic figures (arrows). (Courtesy of Mr. C. A. Farley.)

condition, as indicated in Table 12, but for some consistency the neutral descriptors "sarcomatoid proliferative disease," applied early by Farley (1969a), or the more specific "hemocytic sarcoma" will be applied to those conditions in bivalves which are presumed (without adequate proof, in some instances) to be hemocytic neoplasias and which have the following similarities, as pointed out by Balouet and Poder (1985) and by Farley *et al.* (1986):

- Dense cellular infiltration, especially in connective tissue of the digestive tract, mantle, gonads, and gills
- Cellular abnormalities consisting of an enlarged, irregular nucleus, increased nuclear:cytoplasmic ratios, multiple nucleoli, and frequent mitoses
- Ultramicroscopic anomalies in the form of folded nuclear membranes and deformation of mitochondria
- Absence of phagocytic response when simultaneous infections with protozoan parasites exist
- Absence of *in vitro* aggregation and adhesion to glass, which are characteristic of normal hyaline hemocytes

Table 12. Occurrence of Sarcomatoid Proliferative Disease (Hemocytic Sarcoma) in Bivalve Molluscs

Species Affected	Terminology Used	References
<i>Ostrea lurida</i> (Olympia oyster)	"Vesicular cell neoplasm" and "hematopoietic neoplasm"	Farley and Sparks (1970)
	"Neoplastic disease"	Mix (1975b)
	"Cellular proliferative disorder"	Mix <i>et al.</i> (1977a)
<i>Crassostrea virginica</i> (American oyster)	"Probable neoplastic disease of the hematopoietic system"	Farley (1969a)
	"Hematopoietic neoplasms"	Harshbarger <i>et al.</i> (1979)
<i>Crassostrea gigas</i> (Pacific oyster)	"Probable neoplastic disease of the hematopoietic system"	Farley (1969b)
<i>Ostrea edulis</i> (European flat oyster)	"Hemocytic neoplasms"	Balouet and Poder (1985)
<i>Mytilus edulis</i> (blue mussel)	"Sarcomatoid proliferative disease"	Farley (1969b)
	"Hemic neoplasm"	Mix (1983), Elston <i>et al.</i> (1988a,b)
	"Neoplasm of hematopoietic origin"	Cosson-Mannevy <i>et al.</i> (1984)
	"Proliferating hemocytic condition"	Green and Alderman (1983)
<i>Mya arenaria</i> (soft-shell clam)	"Neoplasms of hematopoietic origin"	Yevich and Barszcz (1976, 1977)
	"Hematopoietic neoplasms" and "sarcoma"	Brown <i>et al.</i> (1977), Reinisch <i>et al.</i> (1984), Farley <i>et al.</i> (1986), Farley (1989)
<i>Cerastoderma edule</i> (cockle)	"Proliferative disorder of possible hemic origin"	Twomey and Mulcahy (1984)
	"Sarcoma, probably of hemocytic origin"	Poder and Auffret (1986), Auffret and Poder (1986)

It should be noted, though, that these criteria may apply as well to neoplasias other than those of hemocytic origin, since other types of cells may be found in invertebrate body fluids.

Balouet and Poder further pointed out that the cause(s) of such presumed hemocytic neoplasia is unknown, except in the case of clams, *Mya arenaria*, in which a retrovirus has been implicated (Cooper and Chang, 1982). In other studies, oil pollution has been suggested as a

possible contributing factor (Farley, 1969a; Yevich and Barszcz, 1977; Mix *et al.*, 1981), but the evidence is conflicting and inconclusive. Mix *et al.* (1982) found a correlation between tissue levels of polycyclic aromatic hydrocarbons and neoplasia in mussels from the Oregon coast—but French studies at the site of the massive *Amoco Cadiz* oil spill of 1978 indicated no relationship between oil pollution and sarcomas in cockles and European flat oysters (Balouet *et al.*, 1986; Poder and Auffret, 1986). Polychlorinated biphenyl (PCB) pollution of growing areas may have been implicated in enhanced prevalences of hemocytic neoplasia in soft-shell clams, (Reinisch *et al.* 1984). The weight of evidence thus far suggests that direct association of molluscan neoplasms with specific pollutants is premature, but that environmental stressors (possibly in concert with an infectious agent) may participate in the development of epizootic levels. Thus far, except for the clam studies mentioned above, viruses have not been demonstrated in neoplastic animals, despite ultrastructural examinations (Auffret and Poder, 1986).

A. Malignant Neoplasms in Soft-Shell Clams

Undoubtedly, the greatest body of literature on invasive neoplasms of bivalve molluscs is concerned with studies of soft-shell clams, *M. arenaria*. The emphasis has been a consequence of (1) interest in the neoplasms as possible biological indicators of oil and PCB pollution and (2) the discovery of epizootic levels of neoplasia in some populations of the species.

A small oil spill at Searsport, Maine, in 1971 provided the impetus for initial histopathological studies which disclosed the presence of neoplasia in clams. Examination of samples from the spill site revealed high prevalences of gonadal tumors (4.3%), characterized by numerous mitotic figures; multinucleated giant cells; lobed irregular nuclei; and invasion of connective tissue surrounding gonadal follicles (Barry and Yevich, 1975). A subsequent study of soft-shell clams from other coastal areas—but especially from another oil-polluted site at Brunswick, Maine—disclosed the presence of a second type of invasive neoplasm, apparently of hematopoietic origin, characterized by large lobed or pleomorphic nuclei, numerous and bizarre mitotic figures, and extensive invasion of connective tissues throughout the body (Yevich and Barszcz, 1976, 1977). A still later survey of ten New England soft-shell clam populations resulted in the findings of neoplasm prevalences of 0–64%, with hematopoietic neoplasia predominating (Brown *et al.*, 1976, 1977). Advanced hematopoietic neoplastic disease was associated in laboratory holding studies, with mortalities.

New data on the course of the disease were provided by Cooper *et al.* (1982a,b). Observations of laboratory-held neoplastic and normal clams indicated that:

- Significantly higher mortalities occurred in neoplastic clams, with survival rates in accord with the severity of the disease.
- The disease followed one of three courses: (1) it was progressive, leading to increased tissue invasion and death (50% of infected individuals), (2) it became chronic (40% of infected individuals), or (3) it diminished in severity or disappeared (10% of infected individuals).

Transmissibility of the hematopoietic neoplasms in soft-shell clams was demonstrated experimentally (Brown, 1980); the author also suggested that environmental stress may have increased susceptibility to neoplasia. Following this study, a B-type retrovirus was reported to have been isolated from neoplastic clams, and the disease was reported to have been reproduced by inoculating purified virus preparations (Appeldoorn and Oprandy, 1980; Oprandy *et al.*, 1981). Patency was achieved 2 months postinoculation. The virus was not, however, identified definitively, nor was it fully characterized. According to a subsequent report, neoplasia was induced and its development was enhanced by 5-bromodeoxyuridine; viral particles isolated from induced neoplastic clams were stated to be similar to those isolated from feral clams—suggesting to the authors (Oprandy and Chang, 1983) that the clam neoplasm-inducing virus may be endogenous in cells of normal clams. These studies suggesting a virus–neoplasm linkage require confirmation and further experimentation, since questions have been raised about methods used to identify neoplasias (*in vitro* diagnosis) and about viral isolations.

Most of the earlier work on soft-shell clam neoplasia resulted from interest in its possible association with oil pollution in northern New England waters. Beginning in the early 1980s, however, interest shifted to epizootics of hematopoietic neoplasms in clams from New Bedford Harbor, Massachusetts, and Chesapeake Bay. In New Bedford Harbor, an area containing high levels of PCBs and other industrial pollutants, prevalences of neoplasms were reported to approach 90% in some samples, as determined by *in vitro* techniques only, and not confirmed histologically (Reinisch *et al.*, 1984). Detectable levels of PCBs were found intracellularly in neoplastic cells from New Bedford clams, but not in hemocytes of normal clams from the same sites, a possible reflection of altered lipid metabolism in neoplastic cells.

A 2½-year survey (1983–1985) of the occurrence of sarcomatous neoplasia in soft-shell clams from Long Island Sound, New York, disclosed

an overall prevalence of slightly over 1%, but with pronounced annual and seasonal variations (Brousseau, 1987). Peak prevalences (as high as 60%) occurred in late fall and winter in some of the sites sampled.

Recent concern about epizootic levels of hematopoietic neoplasia in soft-shell clams from Chesapeake Bay stems from a more immediate problem: the potential for major disease-induced mortalities and their effects on commercial clam populations. The neoplastic disease of clams was not seen in the Bay prior to 1979 and was a rare occurrence until 1983. It first appeared at epizootic levels in December 1983. Prevalences remained high until April 1985. Seasonal progression of disease intensity was seen; light infections in December progressed to advanced and terminal stages by the following April (Farley *et al.*, 1986).

Chronic disease and remissions, as reported by Cooper *et al.* (1982b) from New England clam populations, were not evident in Chesapeake Bay samples. Concurrent laboratory observations indicated 100% mortality of diseased Chesapeake Bay clams from April to June, and field prevalences dropped to 0% in June, presumably because of the deaths of animals with advanced disease. Farley *et al.* (1986) concluded that high prevalences and advancing stages of the disease seen in natural populations may be preludes to significant mortalities in commercial stocks. The authors also suggested an infectious etiology, with the possibility that the infectious agent may have been introduced into the Bay with clams transferred from New England.

The recent (1983–1985) outbreak of sarcomatoid proliferative disease in soft-shell clams from Chesapeake Bay is of particular interest for several reasons:

1. Surveys which began in 1969 indicated that the disease condition did not occur in the population until 1979, but prevalences reached 40% in some 1983 samples and 91% by 1985 (Farley *et al.*, in press). An introduced infectious agent was strongly indicated.
2. Progression of the disease through well-defined stages was followed in field and experimental populations, using new histocytological techniques (Farley *et al.*, 1986). Mortalities were correlated with advanced stages of the disease.
3. Transplantability of the disease was demonstrated by the injection of neoplastic cells; 95% of injected clams developed sarcomas; and advanced infections were lethal in 1–4 months (Farley *et al.*, in press).
4. Data from the clam fishery indicated a severe impact of neoplastic disease on stocks (Farley *et al.*, 1986). A 1-year survey of

one clam population in Chesapeake Bay (Swan Point, October 1984 to October 1985) disclosed an initial high prevalence of the disease, which was followed by high mortalities and a concomitant decline in prevalence. The density of clams and the catch-per-unit effort decreased, and the site became commercially unproductive in the following year (1986). It is interesting and probably relevant that the production of clams in Maryland waters of the Chesapeake Bay declined by 50% from 1983 to 1985, the period encompassed by the epizootic (Farley *et al.*, in press).

B. Malignant Neoplasms in Oysters

Sarcomatous proliferative disease (or hematopoietic neoplasia or hemocytic neoplasia) has been reported in several species of oysters of the genera *Ostrea* and *Crassostrea*. Hemocytic neoplasia, with characteristics like those seen in other bivalves, has been suggested as the cause of recurring mortalities of European flat oysters, *Ostrea edulis*, in Spain and in Yugoslavia (Alderman *et al.*, 1977). Mortalities varied yearly and geographically, but were in the 20-90% range in Yugoslavia and 60-80% in Spain. Oysters sampled from affected areas showed high levels of cellular infiltration of connective tissues, especially in subepithelial areas. The abnormal cells were large, with large basophilic nuclei and reduced cytoplasm. No pathogens were recognized, although other mortalities of flat oysters in Europe have been attributed to two protozoan pathogens (*Marteilia refringens* and *Bonamia ostreae*).

Hemocytic neoplasms have also been reported in *O. edulis* from the French coast (Franc, 1975; Balouet *et al.*, 1986). A 6-year survey (1975-1981) by the latter authors disclosed an overall prevalence of less than 1% in a total sample of about 45,000 oysters. The occurrence of neoplasms was significantly higher in older oysters.

Proliferative diseases also occur in native (Olympia) oysters, *Ostrea lurida*, from the Pacific Northwest (Jones and Sparks, 1969; Farley and Sparks, 1970, Mix *et al.*, 1977a). In the most extensive study (Mix *et al.*, 1977a) prevalences approximated only 2% and no mortalities were attributed to the proliferative disorder.

A cellular proliferative disease similar to that seen in other species of *Ostrea* has been reported (in two of 148 specimens) in *Ostrea chilensis* from Chile (Mix and Breese, 1980).

In the genus *Crassostrea*, Pacific oysters (*Crassostrea gigas*) may be affected by hematopoietic neoplasia. One such case was reported by Farley (1969a) in a specimen from Matsushima Bay, Japan. It is interest-

ing and relevant, though, that no hemocytic neoplasms were found in 8000 *C. gigas* sampled on the French coast during 1975–1981 by Balouet *et al.* (1986).

American oysters (*Crassostrea virginica*) from Chesapeake Bay—12 of 20,000 specimens examined over a 14-year period—were reported to have hematopoietic neoplasms (Harshbarger *et al.*, 1979). These lesions were similar to those reported earlier by Farley (1969a) (five of 30,000 specimens), Couch (1969) (one of 5000 specimens), and Frierman (1976). Cells were relatively large and basophilic, with pleomorphic nuclei; mitoses were common. An important aspect of the Frierman study was that it included examination of over 100 small laboratory-reared inbred populations, two of which exhibited high prevalences of hematopoietic neoplasms. Furthermore, one of these laboratory populations suffered high mortalities that seemed to be directly related to high prevalences of neoplasms in dying oysters, in the absence of other diseases. The study suggested possible genetic differences in susceptibility to neoplasia among the inbred oyster populations.

A sarcomatous lesion, considered to be distinct from the hematopoietic neoplasms, and described as an “undifferentiated sarcoma” was also reported in *C. virginica* from Connecticut waters (one of 1400 specimens examined) by Newman (1972). Characterized by cell and nuclear hypertrophy, large multiple nuclei, abundant mitoses, and irregular nuclear membranes, the neoplasia was disseminated throughout connective tissue and hemolymph. Other specimens of oysters with similar histological appearance were later reported by Harshbarger *et al.* (1979) in Chesapeake Bay (two of 20,000 specimens examined). The lesions, described as “undifferentiated sarcomas,” differed from those described as “hematopoietic neoplasms” in that, in the former, nuclei and nucleoli were much larger, nuclear shape was more irregular, and lesions occurred typically in the gills.

In addition to hematopoietic neoplasms and undifferentiated sarcomas, rare reports of germinomas in *C. virginica* exist. An oyster from Delaware Bay contained an advanced invasive neoplasm consisting of cells originating from germinal epithelium and resembling undifferentiated gametocytes (Farley, 1976b). Two similar germinomas were found in Chesapeake Bay oysters, both with dispersal to connective tissue and blood sinuses (Harshbarger *et al.*, 1979).

C. Malignant Neoplasms in Mussels

A sarcomatoid proliferative disease of mussels, *Mytilus edulis*, from Yaquina Bay, Oregon, was first described by Farley (1969b) (ten of 100

specimens). Characteristics of the disease were similar to those of the hematopoietic neoplasms of oysters and clams: unusually large cells with nuclei two to four times larger than normal hemocytes, abundant mitotic figures with indications of polyploidy, and the presence of aggregations of atypical cells in connective tissue and hemolymph sinuses. Mussels with advanced lesions were emaciated, with degenerative changes suggesting lethality of the disease. A subsequent 5-year study of the disorder (1976–1981) in Yaquina Bay disclosed a seasonal pattern of prevalences (with a peak from January to March), marked geographic variation in disease abundance, and a mean prevalence of 9.8% in the subpopulation with the highest levels of the disease (Mix, 1983). In the words of the author, though, “there was no strong indication that significant mortalities occurred as a result of the condition.” Etiology of the disease was undetermined, although a concurrent study disclosed a statistical association between tissue levels of polynuclear aromatic hydrocarbons and the proliferative disorder (Mix *et al.*, 1979b, 1981). Counterpart studies of the disease in British Columbia (Canada) waters disclosed an overall prevalence of 12.7% in mussels, with epizootic levels of 12–29% in some sampling locations (Cosson-Mannevy *et al.*, 1984).

A recent experimental study of the proliferative disorder in *M. edulis* from a cultured population in Puget Sound, Washington, disclosed that the condition was usually progressive and fatal, but that 20% of the experimental group showed signs of remission (Elston *et al.*, 1988a). An active host response was characterized by hemocytic infiltration, entrapment and sequestering of abnormal cells in a fibrous extracellular matrix apparently secreted by normal hemocytes, and the formation of granuloma-like structures (Elston and Kent, 1988). Transplantation of hemic neoplasms to unaffected mussels was reported, using inoculations of intact neoplastic cells. Transmission by inoculation with cell-free homogenates and by aquarium exposure of disease-free individuals to neoplastic animals was also reported (Elston *et al.*, 1988b).

The proliferative hemocytic condition in *M. edulis* has been reported at low prevalences (0–4.3%) from United Kingdom waters (Lowe and Moore, 1978; Green and Alderman, 1983). In the latter study mussels transplanted from sites at which the disease occurred to sites where it did not occur retained prevalence levels equivalent to those at the site of origin for the 3-year study period.

D. Malignant Neoplasms in Cockles

A proliferative disorder of hemic origin has been reported recently in studies of European cockles, *Cerastoderma edule*, from Ireland and from

France. Frequencies in samples from Cork Harbor, Ireland, were high—41 of 103 specimens (40%) collected from November 1982 to June 1983 (Twomey and Mulcahy, 1984). A later experimental study demonstrated transmission by injecting hemolymph from diseased cockles and also demonstrated 100% mortality in the experimental group by 7 months postinjection (Twomey and Mulcahy, 1988). An extensive sampling of cockles on the northern coast of Brittany, France, disclosed neoplasia in 31 of 752 specimens (4.1%) collected from three shellfish-growing areas from October 1982 to October 1984. Geographic variability was marked, with prevalences at one site as high as 46%, but no disease-related mortalities were observed (Auffret and Poder, 1986; Poder and Auffret, 1986).

E. Epizootic Occurrences of Neoplasms in Bivalves

Prevalences of neoplasms indicative of epizootics have been reported in a number of species and in widely separated locations. A few outbreaks have been well documented:

- Proliferative sarcomatoid neoplasms have been seen in blue mussels, *Mytilus edulis*, from Yaquina Bay, Oregon (Farley and Sparks, 1970; Cosson-Mannevy *et al.*, 1984), and from the British coast (Green and Alderman, 1983).
- Gill carcinomas at epizootic levels have been seen in local populations of duck clams, *Macoma balthica*, from Chesapeake Bay (Christensen *et al.*, 1974; Farley, 1976b).
- Germinomas and hemocytic sarcomas have been seen in soft-shell clams, *Mya arenaria*, from several locations on the New England coast in the 1970s (Brown *et al.*, 1977; Yevich and Barszcz, 1977), and the hemocytic sarcomas have been subsequently reported at epizootic levels in clams from New Bedford Harbor, Massachusetts, and Chesapeake Bay (Reinisch *et al.*, 1984; Farley, *et al.*, 1986).

The epizootics have a number of features in common, as was pointed out by Farley (1976b) and by Farley *et al.* (1986):

1. Prevalences at some localized sites may reach 90%.
2. Neoplasms are common from October through April and seem to progress during the winter.
3. Advanced cases, characterized by dense aggregations of neoplastic cells throughout the blood sinuses and connective tissues, are lethal.
4. The neoplasms are invasive, and cells are highly anaplastic.

5. Within a single species the appearance of neoplastic cells is identical.

Not all epizootic neoplasms of bivalves are hemocytic sarcomas—or even sarcomas. Neoplasms characterized by masses of undifferentiated cells in vascular spaces of the duck clam, *Macoma balthica*, from Chesapeake Bay were shown by electron microscopy to be of epithelial origin—hence, “carcinomas” (Farley, 1976a; Farley *et al.*, 1986). The neoplastic condition in *M. balthica* was studied earlier by Christensen *et al.* (1974). Prevalences as high as 10% were recorded in some samples. The disease was highly invasive—moving from initial foci in the gill epithelium, across basement membranes, to produce diffuse systemic disseminations of neoplastic cells. Despite the presence of a protistan pathogen (*Perkinsus* sp.), mortalities attributable to the neoplastic disease were observed in laboratory holding experiments, using clams from wild populations.

Gonadal neoplasms (germinomas) have been reported in soft-shell clams, *Mya arenaria*, and (less certainly) from hard-shell clams, *Mercenaria mercenaria*, from the New England coast (Barry and Yevich, 1975; Yevich and Barszcz, 1976, 1977). One report (Barry and Yevich, 1975) described invasive gonadal tumors in 5% of the soft-shell clams examined 4 months after an oil spill on the Maine coast. Similar tumors were not found in clams from eight reference sites elsewhere in New England. Gonadal neoplasms were differentiated from hematopoietic neoplasms by the presence of smaller cells with more clumped chromatin (Brown *et al.*, 1977).

Gonadal neoplasms were also reported in hard-shell clams, *M. mercenaria*, from Narragansett Bay, Rhode Island (Yevich and Barry, 1969). Tumor cells proliferated from the germinal epithelium into the follicle lumen and in one case were reported to have invaded the kidney. A later study disclosed similar gonadal neoplasia in 14 of 539 clams sampled over a 2-year period (Barry and Yevich, 1972). Gonadal neoplasms were also reported in hard-shell clams sampled in Florida (Hesselman *et al.*, 1987). Sampling over an 18-month period in the Indian River in 1985 and in 1986 disclosed an overall prevalence of 12%.

F. Benign Tumors in Bivalves

Other than the recent flurry of papers concerned with malignant tumors of probable gonadal or hemocytic origin, the literature on tumors in marine molluscs consists principally of a century of reports on the rare occurrence of usually benign growths in individual animals. The list includes mesenchymal tumors of oysters (*Crassostrea virginica*, *C. gigas*,

Pinctada margaritifera, and *Saccostrea commercialis*), epitheliomas of oysters (*S. commercialis* and *C. gigas*), as well as rare myofibromas and neurofibromas of oysters (*C. gigas*). Of these, the papillary epitheliomas of Australian oysters, *S. commercialis*, are of the greatest interest, since they occur in varying (but low) prevalences wherever commercial populations of *S. commercialis* exist in eastern Australia. Multiple tumors with a cauliflower-like appearance arise from the mantle and the gills, and at times the neoplasms invade other body tissues (Wolf, 1969, 1976). An invasive epithelioma was also reported by Pauley and Sayce (1972) in *C. gigas*. The neoplasm was similar to the papillary epitheliomas of Australian oysters and may have arisen from the periostracal groove and gland.

Reports of most other types of tumorous growths are usually based on single specimens, and often there is some question as to whether the growths are benign tumors or hyperplastic responses to trauma. Ryder (1887) published the earliest report of a tumor in oysters, describing a nodular growth over 3 cm in greatest dimension in the pericardial cavity of *C. virginica*. The tumor seemed to originate from connective tissue surrounding the rectum or from the pericardial membranes. Smith (1934) described a stalked mesenchymal tumor arising from the outer surface of the pericardium in the same species; he considered the tumor to be similar to that reported by Ryder. Still other tumors associated with the heart or the pericardium of oysters were reported by Dinamani and Wolf (1973) and by Mix and Riley (1977)—the former a stalked tumor in the pericardial cavity of the Australian rock oyster, *S. commercialis*, and the latter a multilobed tumor arising from auricle tissue of the heart.

A stalked mesenchymal tumor in *C. gigas* was reported by Sparks and Pauley (1963, 1964) and by Sparks *et al.* (1964). The growth was located posterior to the rectum and appeared to originate from the mantle in the region of the rectum. Still another stalked mesenchymal tumor originating from the body mass was reported by Pauley *et al.* (1965). The neoplasm was elongate and ovoid and had an irregular nodular surface. Other tumorous growths on Pacific oysters, one apparently originating from the adductor muscle, were described by Pauley and Sayce (1968), Sparks *et al.* (1969), and Harshbarger (1976). An internal fibrous tumor, considered to originate from smooth muscle, was also described by Pauley and Sayce (1968). The lesion consisted of a stroma of elongate, fibrous connective tissue with a hollow center. The tumor margin was well defined and noninvasive, and no mitotic figures were seen. In a companion paper (Pauley *et al.*, 1968) a second internal tumor, consisting of a proliferation of collagen and nerve tissue, was reported. The tumor was invasive, having replaced much of the connective tissue nor-

mally found in the affected area, and an intense inflammatory response was associated with it. The authors identified the tumor tentatively as a ganglioneuroma.

Mesenchymal tumors have been reported from pearl oysters, *P. margaritifera*, from Australia (Dix, 1972). Two stalked polyplike growths originated from the visceral mass; they were classified by the author as benign fibrovascular polyps.

Hueper (1963) reported cauliflower-like papillary tumors at the anterior end of soft-shell clams collected from Chesapeake Bay. He termed the condition "endemic" and reported it in about 2% of clams collected from certain Bay areas. Hyperplastic polypoid and papillary lesions were reported by Taylor and Smith (1966) in the foot of the gaper clam, *Tresus nuttalli*. One such fleshy growth measured 4×7 mm. Histologically, the area was characterized by intense inflammatory reaction with infiltration of leukocytes and fibroblasts. A polypoid tumor of the foot of the butter clam, *Saxidomus giganteus*, was reported by Pauley (1967). The growth was composed primarily of histologically normal muscle tissue covered by convoluted columnar epithelium, without mitotic figures or an inflammatory response.

A few tumors have been recognized and reported in squids. Jullien (1928) described as tumors certain protuberances and inflammations in the mouths of two squids, *Sepia officinalis*. Later, Jullien and Jullien (1951) found two other squids with extensive benign tumors on the ventral side of the visceral mass. In each case the lesions were characterized by leukocytic infiltration, disruption of the epidermis, and a lack of vascularization at the center of the tumor. They were considered to be of connective tissue origin.

In summary, the recent literature on tumors in molluscs is dominated by reports of malignant neoplasms in bivalves—especially a so-called "sarcomatous proliferative disorder of probable hematopoietic origin" seen, sometimes at epizootic levels, in clams, oysters, cockles, and mussels. The presumed neoplastic condition is characterized by "invasive dissemination of enlarged, atypical, proliferative, undifferentiated cells with cytologic and mitotic abnormalities" (Cosson-Mannevy *et al.*, 1984). The etiology of the disease in most bivalves is unknown, although viruses, environmental pollutants, biotoxins, and parasitic infections have been considered. Only in soft-shell clams, in which an infectious process is strongly indicated, has a tentative association with viral infection been made, and even here additional experiments are needed. An early proposed relationship of neoplasia and environmental pollution has not been substantiated. Considering all of the surveys conducted and the several suggestions of association of neoplasia with

petroleum hydrocarbon and other types of pollution (Yevich and Barszcz, 1977; Mix *et al.*, 1981; Reinisch *et al.*, 1984), it seems that there are as many or more examples of high prevalences *not* related to hydrocarbons or other known pollutants. The role of an environmental stressor cannot, of course, be dismissed. An alternative hypothesis is that this is a true neoplastic disorder—the invertebrate analog or homolog of malignant neoplasia in the vertebrates (Mix, 1975b, 1976, 1979).

Although the sarcomas reported from various bivalves have generally similar characteristics, subtle differences occur from species to species. For example, mussel and oyster neoplastic cells have polymorphic lobulated nuclei (Farley and Sparks, 1970; Mix *et al.*, 1979a), whereas nuclei of cockle neoplastic cells are oval or kidney shaped, with regular contours (Auffret and Poder, 1986). These observations, combined with reports of high prevalences in one bivalve species and the absence of the disease in other bivalves inhabiting the same site (Christensen *et al.*, 1974; Mix *et al.*, 1977a), suggest that susceptibility and responses to causative agent(s) are variable. An important unifying observation in several species is that the hemocytic sarcoma has been demonstrated to cause mortalities, and, in the case of soft-shell clams, to affect population abundance.

Other than the hemocytic sarcomas, malignant neoplasias in bivalve molluscs consist of germinomas in clams and in oysters and (marginally) a papillary epithelioma, with a tendency toward invasiveness, in Australian oysters. Benign tumors are varied but rare, such that individual cases have been reported in a sporadic literature spanning 100 years.

III. NEOPLASTIC DISEASES OF CRUSTACEANS

Reports of tumors in crustaceans of economic importance are so rare that any reviewer is tempted to reach for incidental observations of gross abnormalities, for rare descriptions of "idiopathic" hyperplasias or for parasite-induced cell proliferations—few, if any, of which would fit a strict definition of neoplasia. The paucity of information may be due in large part to inadequate histopathological studies (a situation which is changing with the recent interest in diseases of cultured shrimp, lobsters, crabs, and other crustaceans), but it seems likely that the scarcity of reports should reflect, to at least some small extent, the actual scarcity of abnormalities.

Early reports of tumorlike growths in Crustacea include a tumor in a lobster, *Homarus americanus*. The growth originated in the stomach wall, pushed through the carapace behind the eyes, became enlarged, and

finally killed the animal (Prince, 1897). This, as was pointed out in a review of tumors in invertebrates by Scharrer and Lochhead (1950), was probably the earliest observation of a presumptive tumorous growth in a crustacean. Several other reports concern a probable inflammatory response to injury in a lobster (Herrick, 1895, 1909) and probable parasite-induced hyperplasia in a crab (Fischer, 1928).

Several more recent reports help to maintain what is, at best, a feeble flow of literature on crustacean tumors. A brown shrimp, *Penaeus aztecus*, reared in an experimental aquaculture facility in Texas, was found with a cauliflower-like lateral growth on the abdomen (Sparks and Lightner, 1973). Histologically, the growth was covered by cuticle, and the core consisted of hyperplastic tissue constituents, especially connective tissue. No mitotic figures were seen; the authors suggested that the abnormality was a benign neoplasm.

Nonneoplastic growths described as "hamartomas" (defined as excessive overgrowths of normal cells and tissues in an organ or tissue composed of identical cellular elements) were observed in 33 postlarval brown shrimp (*P. aztecus*) and white shrimp (*Penaeus setiferus*) in a polluted Mississippi estuary (Overstreet and Van Devender, 1978). The often conspicuous ventrally protruding growths from the abdomen consisted of muscle tissue surrounded by a thin cuticle. Prevalences during a 3-year sampling period were 0.08% (20 of 26,238) in brown shrimp and 0.56% (13 of 2320) in white shrimp. The growths, described as tumors only in the broad definition of the term, were considered by the authors to be associated with pollution, since 93.9% of the abnormal shrimp were taken from a harbor which received domestic wastes.

Another unusual proliferative abnormality of the midgut and the ventral nerve cord of cultured postlarval, juvenile, and adult Kuruma prawns (*Penaeus japonicus*) was described recently by Lightner *et al.* (1984). Characterized by thickening of the midgut basement membrane and hyperplasia of the nerve cord sheath, the disorder (labeled "gut and nerve syndrome"), occurred in most populations of *P. japonicus* imported to Hawaii from Japan or reared from those imports. Gross signs of the disease included poor growth, lethargy, and abdominal muscle necrosis. Eventual 100% mortality of affected populations reared in high-density systems due to this chronic debilitating condition was reported by the authors, who also suggested that successful intensive culture of Kuruma prawn in Hawaii may depend on understanding and control of the disease.

The most recent example of proliferative disease in crustaceans is probably the best: a hematopoietic sarcoma in the white shrimp, *Penaeus vannamei* (Lightner and Brock, 1987). The specimen, from an experimen-

tal culture facility in Hawaii, was identified during routine histopathological examination for viral diseases. The neoplasm originated in hematopoietic nodules ventral and lateral to the nerve cord. Lymphoid-like cells in hematopoietic lesions were enlarged, poorly differentiated and mitotically active, with enlarged nuclei and multiple nucleoli. Foci of such neoplastic cells also occurred in the gills, subcuticular tissues, hemocoel, striated muscle, and loose connective tissue. Histopathological study also disclosed the presence of intranuclear inclusions in the neoplastic hematopoietic tissue and in other tissues; these inclusions were of the type diagnostic of viral infection (infectious hypodermal and hematopoietic necrosis, or IHHN, virus), suggesting—as was pointed out by Lightner and Brock—“a possible role for chronic IHHN infection in the development of the neoplastic lesions in this animal.” At any rate, and in agreement with the authors, the observed characteristics of the lesions indicate a malignant neoplasm: a hematopoietic sarcoma.

These, then, are the tumors and tumorlike abnormalities of marine Crustacea of economic importance, as recognized to the present. It seems probable, though, that expanded histopathological scrutiny in future studies will reveal additional neoplasias that are not apparent grossly—as demonstrated in recent research with molluscs. The report by Lightner and Brock (1987) gives a clue that this may happen soon.

IV. CONCLUSIONS

Reports of neoplasms in molluscs and in crustaceans have increased in frequency during the past decade, especially with the discovery of epizootic sarcomas in bivalves and with concomitant indications of lethality of the disease. Examination of the literature leads to a number of conclusions and speculations:

1. The early anemic literature on presumed tumors in molluscs and in crustaceans was confined to reports of gross abnormalities; more recent histopathological studies—designed primarily to study infectious diseases—have disclosed the existence of malignant neoplasms with no distinctive external signs.
2. There are already indications that some of the neoplasms in bivalves are lethal, and that they may occur at epizootic levels, resulting in effects on the abundance of local populations. Following this line of reasoning, malignant neoplasms may prove to be an important and hitherto unrecognized mortality factor in invertebrate populations.

3. The etiologies of most molluscan and crustacean neoplasms are still unknown. Early suggestions of a possible association of hemocytic sarcomas of bivalves with petroleum hydrocarbon pollution have not been substantiated as more extensive sampling has been done. An infectious process has been strongly suggested in some studies of epizootic neoplasms, but presumptive evidence for possible viral involvement in tumor induction has been reported only for hemocytic sarcomas of the soft-shell clam and the white shrimp (Oprandy *et al.*, 1981; Lightner and Brock, 1987).
4. The genetic background for neoplasia in invertebrates is largely unknown, but interesting insights have been reported. One is the finding of remarkably high prevalences of hemocytic sarcomas in only two of more than 100 inbred populations of oysters (Frierman, 1976); another is an indication of the reduced frequency of lethal hemocytic sarcomas in clam populations in which the disease is enzootic, when compared with populations in which the disease may have been recently introduced (Farley *et al.*, 1986).

It seems clear, then, that research on neoplasia in molluscs and in crustaceans has moved from an earlier phase of descriptions of grossly recognizable (and mostly benign) tumors to the current phase of histopathological studies of malignant neoplasms, in many instances with concurrent field and laboratory observations. Strong motivations exist for expansion of such studies: elucidation of the role that neoplasms may play in mortalities, and the continued exploration of the relationship of environmental stressors (especially pollutants) and neoplastic disease.

REFERENCES

- Alderman, D. J., van Banning, P., and Perez-Colomer, A. (1977). Two European oyster (*Ostrea edulis*) mortalities associated with an abnormal hemocytic condition. *Aquaculture* **10**, 335–340.
- Appeldoorn, R. S., and Oprandy, J. J. (1980). Tumors in soft-shell clams and the role played by a virus. *Maritimes* **24**(1), 4–6.
- Auffret, M., and Poder, M. (1986). Sarcomatous lesion in the cockle *Cerastoderma edule*. II. Electron microscopical study. *Aquaculture* **58**, 9–15.
- Balouet, G., and Poder, M. (1978). Hyperplasie hémocytaire chez *Ostrea edulis* L. *Haliotis* **9**, 99–102.
- Balouet, G., and Poder, M. (1979). A proposal for classification of normal and neoplastic types of blood cells in molluscs. In "Advances in Comparative Leukemia Research" (D. S. Yohn, B. A. Lapin, and J. R. Blakeslee, eds.), pp. 205–208. Elsevier/North-Holland, Amsterdam.

- Balouet, G., and Poder, M. (1985). A consideration of the cellular reactions in bivalve molluscs, with emphasis on haemocytic diseases. In "Fish and Shellfish Pathology" pp. 381-385. (A. E. Ellis, ed.), Academic Press, London.
- Balouet, G., Poder, M., and Cahour, A. (1982). Hyaline cell proliferation in *Ostrea edulis* L.: Histogenetic and oncologic approach. *Proc. Int. Colloq. Invertebr. Pathol.*, 3rd pp. 268-270.
- Balouet, G., Cahour, A., and Auffret, M. (1983). Haemocyctosarcomas in marine bivalve molluscs: Hypothesis on histogenesis in European flat oyster *Ostrea edulis* L. *Leuk. Rev.* **1**, 26-31.
- Balouet, G., Poder, M., Cahour, A., and Auffret, M. (1986). Proliferative hemocytic condition in European flat oyster (*Ostrea edulis*) from Breton coasts: A six-year survey. *J. Invertebr. Pathol.* **48**, 208-215.
- Barry, M. M., and Yevich, P. P. (1972). Incidence of gonadal cancer in the quahog, *Mercentaria mercenaria*. *Oncogenesis* **26**, 96-97.
- Barry, M. M., and Yevich, P. P. (1975). The ecological, chemical and histopathological evaluation of an oil spill site. Part III. Histopathological studies. *Mar. Pollut. Bull.* **6**, 171-173.
- Brousseau, D. J. (1987). Seasonal aspects of sarcomatous neoplasia in *Mya arenaria* (soft-shell clam) from Long Island Sound. *J. Invertebr. Pathol.* **50**, 269-276.
- Brown, R. S. (1980). The value of the multidisciplinary approach to research on marine pollution effects as evidenced in a three-year study to determine the etiology and pathogenesis of neoplasia in the soft-shell clam, *Mya arenaria*. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **179**, 125-128.
- Brown, R. S., Wolke, R. E., Saila, S. B. (1976). A preliminary report on neoplasia in feral populations of the soft-shell clam *Mya arenaria*: Prevalence, histopathology and diagnosis. *Proc. Int. Collq. Invertebr. Pathol.*, 9th pp. 151-158.
- Brown, R. S., Wolke, R. E., Saila, S. B., and Brown, C. W. (1977). Prevalence of neoplasia in 10 New England populations of the soft-shell clam (*Mya arenaria*). *Ann. N.Y. Acad. Sci.* **298**, 522-534.
- Brown, R. S., Wolke, R. E., Brown, C. W., and Saila, S. B. (1979). Hydrocarbon pollution and the prevalence of neoplasia in New England soft-shell clams (*Mya arenaria*). In "Animals as Monitors of Environmental Pollutants," pp. 41-51. *Natl. Acad. Sci., Washington, D.C.*
- Christensen, D. J., Farley, C. A., and Kern, F. G. (1974). Epizootic neoplasms in the clam *Macoma balthica* (L.) from Chesapeake Bay. *J. Natl. Cancer Inst.* **52**, 1739-1749.
- Cooper, K. R., and Chang, P. W. (1982). A review of the evidence supporting a viral agent causing an haematopoietic neoplasm in the soft shell clam *Mya arenaria*. *Proc. Int. Colloq. Invertebr. Pathol.*, 15th pp. 271-272.
- Cooper, K. R., Brown, R. S., and Chang, P. W. (1982a). Accuracy of blood cytological screening techniques for the diagnosis of a possible hematopoietic neoplasm in the bivalve mollusk, *Mya arenaria*. *J. Invertebr. Pathol.* **39**, 281-289.
- Cooper, K. R., Brown, R. S., and Chang, P. W. (1982b). The course and mortality of a hematopoietic neoplasm in the soft-shell clam, *Mya arenaria*. *J. Invertebr. Pathol.* **39**, 149-157.
- Cosson-Mannevy, M. A., Wong, C. S., and Cretney, W. J. (1984). Putative neoplastic disorders in mussels (*Mytilus edulis*) from southern Vancouver Island waters, British Columbia. *J. Invertebr. Pathol.* **44**, 151-160.
- Couch, J. A. (1969). An unusual lesion in the mantle of the American oyster, *Crassostrea virginica*. *Natl. Cancer Inst. Monogr.* **31**, 557-562.
- Couch, J. A., and Winstead, J. T. (1979). Concurrent neoplastic and protistan disorders in the American oyster (*Crassostrea virginica*). *Haliotis* **8**, 249-253.

- Dawe, C. J. (1968). Invertebrate animals in cancer research. *Gann Monogr.* **5**, 45–55.
- Dinamani, P., and Wolf, P. H. (1973). Multiple tumors in the pericardial cavity of an Australian rock oyster, *Crassostrea commercialis* (Iredale and Roughley). *Int. J. Cancer* **11**, 293–299.
- Dix, T. G. (1972). Two mesenchymal tumors in a pearl oyster, *Pinctada margaritifera*. *J. Invertebr. Pathol.* **20**, 317–320.
- Elston, R. A., and Kent, M. L. (1988). Biology of the hemic neoplasia in the bay mussel, *Mytilus edulis*. *Prog. Abstr. Annu. Meet. Soc. Invertebr. Pathol.*, 21st p. 42 (abstract).
- Elston, R. A., Allen, S. K., Radnay, E. W., and Kent, M. L. (1987). Experimental studies on hemic proliferative disease in the mussel, *Mytilus edulis*. *Prog. Abstr. Annu. Meet. Soc. Invertebr. Pathol.*, 20th p. 85 (abstract).
- Elston, R. A., Kent, M. L., and Drum, A. S. (1988a). Progression, lethality, and remission of hemic neoplasia in the bay mussel, *Mytilus edulis*. *Dis. Aquat. Org.* **4**, 135–142.
- Elston, R. A., Kent, M. L., and Drum, A. S. (1988b). Transmission of hemic neoplasia in the bay mussel, *Mytilus edulis*, using whole cells and cell homogenate. *Dev. Comp. Immunol.* **12**, 719–729.
- Farley, C. A. (1969a). Probable neoplastic disease of the hematopoietic system in oysters, *Crassostrea virginica* and *Crassostrea gigas*. *Natl. Cancer Inst. Monogr.* **31**, 541–555.
- Farley, C. A. (1969b). Sarcomatoid proliferative disease in a wild population of blue mussels (*Mytilus edulis*). *J. Natl. Cancer Inst.* **43**, 509–516.
- Farley, C. A. (1976a). Proliferative disorders in bivalve mollusks. *Mar. Fish. Rev.* **38**(10), 30–33.
- Farley, C. A. (1976b). Ultrastructural observations on epizootic neoplasia and lytic virus infection in bivalve mollusks. *Prog. Exp. Tumor Res.* **20**, 283–294.
- Farley, C. A. (1977). Neoplasms in estuarine mollusks and approaches to ascertain causes. *Ann. N.Y. Acad. Sci.* **298**, 225–232.
- Farley, C. A. (1989). Selected aspects of neoplastic progression in molluscs. In "Progressive Stages of Malignant Growth/Development. Vol. I, Part 5: Comparative Aspects of Tumor Progression" (H. E. Kaiser, ed), pp. 24–31. Nijhoff, New York.
- Farley, C. A., and Sparks, A. K. (1970). Proliferative diseases of hemocytes, endothelial cells, and connective tissue cells in mollusks. *Bibl. Haematol.* **36**, 610–617.
- Farley, C. A., Otto, S. V., and Reinisch, C. L. (1986). New occurrence of epizootic sarcoma in Chesapeake Bay soft shell clams (*Mya arenaria*). *Fish. Bull.* **84**, 851–857.
- Farley, C. A., Plutschak, D. L., and Scott, R. F. Epizootiology and distribution of transmissible sarcoma in Maryland soft-shell clams, *Mya arenaria*, 1984–1988. *Envir. Health Persp.* (in press).
- Fischer, E. (1928). Association chez le crabe d'un tissu parasite et d'une trame conjunctive analogue à certains processus tumoraux. *Bull. Assoc. Fr. Étude Cancer* **17**, 468–470.
- Franc, A. (1975). Hyperplasie hémocytaire et lésions chez l'huître plate (*Ostrea edulis* L.). *C. R. Hebd. Séances Acad. Sci., Ser. D* **280**, 495–498.
- Frierman, E. M., and Andrews, J. D. (1976). Occurrence of hematopoietic neoplasms in Virginia oysters. *J. Natl. Cancer Inst.* **56**, 319–324.
- Green, M., and Alderman, D. J. (1983). Neoplasia in *Mytilus edulis* from United Kingdom waters. *Aquaculture* **30**, 1–10.
- Harshbarger, J. C. (1976). Descriptions of polyps and epidermal papillomas in three bivalve mollusk species. *Mar. Fish. Rev.* **38**(10), 25–29.
- Harshbarger, J. C., Otto, S. V., and Chang, S. C. (1979). Proliferative disorders in *Crassostrea virginica* and *Mya arenaria* from the Chesapeake Bay and intranuclear virus-like inclusions in *Mya arenaria* with germinomas from a Maine oil spill site. *Haliotis* **8**, 243–248.

- Herrick, F. H. (1895). The American lobster: A study of its habits and development. *Bull. U.S. Fish Comm.* **15**, 252 pp.
- Herrick, F. H. (1909). Natural history of the American lobster. *Bull. U.S. Bur. Fish.* **29**, 149–408.
- Hesselman, D. M., Peters, E. C., and Blake, N. J. (1987). Neoplasia in the hard shell clam (genus *Mercenaria*). *Prog. Abstr. Annu. Meet. Soc. Invertebr. Pathol.*, 20th (abstract).
- Hueper, W. C. (1963). Environmental carcinogenesis in man and animals. *Ann. N.Y. Acad. Sci.* **108**, 963–1038.
- Jones, E., and Sparks, A. K. (1969). An unusual histopathological condition in *Ostrea lurida* from Yaquina Bay, Oregon. *Proc. Natl. Shellfish. Assoc.* **59**, 11.
- Jullien, A. (1928). De certaines tumeurs et inflammations du manteau de la seiche. *Arch. Zool. Exp. Gen.* **67**, 139–158.
- Jullien, A., and Jullien, A. P. (1951). Sur un type de tumeur non provoquée expérimentalement et observée chez la seiche. *C. R. Acad. Sci.* **233**, 1322–1324.
- Lauckner, G. (1983). Diseases of Mollusca: Bivalvia. In "Diseases of Marine Animals" (O. Kinne, ed.), Vol. II, Chap. 13. Biol. Anst. Helgol., Hamburg, FRG.
- Lightner, D. V., and Brock, J. A. (1987). A lymphoma-like neoplasm arising from hemopoietic tissue in the white shrimp, *Penaeus vannamei* Boone (Crustacea: Decapoda). *J. Invertebr. Pathol.* **49**, 188–193.
- Lightner, D. V., Redman, R. M., Bell, T. A., and Brock, J. A. (1984). An idiopathic proliferative disease syndrome of the midgut and ventral nerve in the Kuruma prawn, *Penaeus japonicus* Bate, cultured in Hawaii. *J. Fish Dis.* **7**, 183–191.
- Lowe, D. M., and Moore, M. N. (1978). Cytology and quantitative cytochemistry of a proliferative atypical hemocytic condition in *Mytilus edulis* (Bivalvia, Mollusca). *J. Natl. Cancer Inst.* **60**, 1455–1459.
- Mix, M. C. (1975a). The neoplastic disease of Yaquina Bay bivalve mollusks. In "The Cell Cycle in Malignancy and Immunity" (J. C. Hampton, ed.), pp. 369–386. Natl. Tech. Inf. Serv., Springfield, Virginia.
- Mix, M. C. (1975b). Proliferative characteristics of atypical cells in native oysters (*Ostrea lurida*) from Yaquina Bay, Oregon. *J. Invertebr. Pathol.* **26**, 289–298.
- Mix, M. C. (1976). A review of the cellular proliferative disorders of oysters (*Ostrea lurida*) from Yaquina Bay, Oregon. *Prog. Exp. Tumor Res.* **20**, 275–282.
- Mix, M. C. (1979). Chemical carcinogens in bivalve mollusks from Oregon estuaries. *U.S. Environ. Prot. Agency, Ecol. Res. Serv.* **EPA-600/3-79-034**.
- Mix, M. C. (1982). Polynuclear aromatic hydrocarbons and cellular proliferative disorders in bivalve molluscs from Oregon estuaries. *U.S. Environ. Prot. Agency, Natl. Tech. Inf. Serv.* **EPA-600/4-82-026**, 26 pp.
- Mix, M. C. (1983). Haemic neoplasms of bay mussels, *Mytilus edulis* L. from Oregon: Occurrence, prevalence, seasonality and histopathological progression. *J. Fish Dis.* **6**, 239–248.
- Mix, M. C. (1986). Cancerous diseases in aquatic animals and their association with environmental pollutants: A critical literature review. *Mar. Environ. Res.* **20**, 1–141.
- Mix, M. C., and Breese, W. P. (1980). A cellular proliferative disorder in oysters (*Ostrea chilensis*) from Chiloe, Chile, South America. *J. Invertebr. Pathol.* **36**, 123–124.
- Mix, M. C., and Riley, R. T. (1977). A pericardial tumor in a native (Olympia) oyster, *Ostrea lurida*, from Yaquina Bay, Oregon. *J. Invertebr. Pathol.* **30**, 104–107.
- Mix, M. C., Pribble, H. J., Riley, R. T., and Tomasovic, S. P. (1977a). Neoplastic disease in bivalve mollusks from Oregon estuaries with emphasis on research on proliferative disorders in Yaquina Bay oysters. *Ann. N.Y. Acad. Sci.* **298**, 356–373.
- Mix, M. C., Riley, R. T., King, K. I., Trenholm, S. R., and Schaffer, R. L. (1977b). Chemical

- carcinogens in the marine environment. Benzo(a)pyrene in economically-important bivalve mollusks from Oregon estuaries. In "Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems" (D. A. Wolfe, ed.), Pergamon, New York.
- Mix, M. C., Hawkes, J. W., and Sparks, A. K. (1979a). Observation on the ultrastructure of large cells associated with putative neoplastic disorders of mussels, *Mytilus edulis*, from Yaquina Bay, Oregon. *J. Invertebr. Pathol.* **34**, 41–56.
- Mix, M. C., Trenholm, S. R., and King, K. I. (1979b). Benzo(a)pyrene body burdens and the prevalence of cellular proliferative disorders in mussels, *Mytilus edulis*, from Yaquina Bay, Oregon. In "Pathobiology of Environmental Pollutants—Animal Models and Wildlife as Monitors." Natl. Acad. Sci., Washington, D.C.
- Mix, M. C., Schaffer, R. L., and Hemingway, S. J. (1981). Polynuclear aromatic hydrocarbons in bay mussels (*Mytilus edulis*) from Oregon. In "Phyletic Approaches to Cancer" (C. J. Dawe, J. C. Harshbarger, S. Kondo, T. Sugimura, and S. Takayama, eds), pp.167–177. Jpn. Sci. Soc. Press. Tokyo.
- Mix, M. C., Hemingway, S. J., and Schaffer, R. L. (1982). Benzo(a)pyrene concentrations in somatic and gonad tissues of bay mussels, *Mytilus edulis*. *Bull. Environ. Contam. Toxicol.* **28**, 46–51.
- Newman, M. W. (1972). An oyster neoplasm of apparent mesenchymal origin. *J. Natl. Cancer Inst.* **48**, 237–243.
- Oprandy, J., and Chang, P. W. (1983). 5-Bromodeoxyuridine induction of haematopoietic neoplasia and retrovirus activation of the soft shell clam *Mya arenaria*. *J. Invertebr. Pathol.* **42**, 196–206.
- Oprandy, J. J., Chang, P. W., Pronovost, A. D., Cooper, K. R., Brown, R. S., and Yates, V. J. (1981). Isolation of a viral agent causing hemotopoietic neoplasia in the soft-shell clam, *Mya arenaria*. *J. Invertebr. Pathol.* **38**, 45–51.
- Otto, S. V., and Farley, C. A. (1976). Neoplasms in bivalve mollusks from the upper Chesapeake Bay and adjoining tributaries. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st p. 159.
- Overstreet, R. M., and Van Devender, T. (1978). Implication of an environmentally induced hamartoma in commercial shrimps. *J. Invertebr. Pathol.* **31**, 234–238.
- Pauley, G. B. (1967). A butter clam (*Saxidomus giganteus*) with a polypoid tumor on the foot. *J. Invertebr. Pathol.* **9**, 577–579.
- Pauley, G. B. (1969). A critical review of neoplasia and tumor-like lesions in mollusks. *Natl. Cancer Inst. Monogr.* **31**, 509–539.
- Pauley, G. B., and Sayce, C. S. (1968). An internal fibrous tumor in a Pacific oyster. *Crassostrea gigas*. *J. Invertebr. Pathol.* **10**, 1–8.
- Pauley, G. B., and Sayce, C. S. (1972). An invasive epithelial neoplasm in a Pacific oyster, *Crassostrea gigas*. *J. Natl. Cancer Inst.* **49**, 897–902.
- Pauley, G. B., Sparks, A. K., and Chew, K. K. (1965). Studies in oyster pathology. Research in fisheries, 1964. *Univ. Wash., Coll. Fish., Contrib.* **184**, 53–54.
- Pauley, G. B., Sparks, A. K., and Sayce, C. S. (1968). An unusual internal growth associated with multiple watery cysts in a Pacific oyster (*Crassostrea gigas*). *J. Invertebr. Pathol.* **11**, 398–405.
- Poder, M., and Auffret, M. (1986). Sarcomatous lesion in the cockle *Cerastoderma edule*. I. Morphology and population survey in Brittany, France. *Aquaculture* **58**, 1–8.
- Poder, M., Auffret, M., and Balouet, G. (1983). Études pathologiques et épidémiologiques des lésions parasitaires chez *Ostrea edulis* L. Premiers résultats d'une recherche prospective comparative chez les principales espèces de Mollusques des zones ostréicoles de Bretagne Nord. Colloque sur les Bases Biologiques de l'Aquaculture, Montpellier. IFREMER (Institut Français de Recherche pour l'Exploitation de la Mer) Actes Colloq. **1**, 125–138.

- Prehn, R. T. (1975). Neoplasia. In "Principles of Pathobiology" (M. F. La Via and R. B. Hill, Jr., eds.), 2nd ed., pp. 203-254. Oxford University Press, London.
- Prince, E. E. (1897). Special report on the natural history of the lobster. *Annu. Rep., Dep. Mar. Fish. Can.*, 29th, Suppl. 1, Parts I-IV 36 pp.
- Reinisch, C. L., Charles, A. M., and Troutner, J. (1983). Unique antigens on neoplastic cells of the soft shell clam *Mya arenaria*. *Dev. Comp. Immunol.* **7**, 33-39.
- Reinisch, C. L., Charles, A. M., and Stone, A. M. (1984). Epizootic neoplasia in soft shell clams collected from New Bedford Harbor. *Hazard. Waste* **1**, 73-81.
- Ryder, J. A. (1887). On a tumor in the oyster. *Proc. Natl. Acad. Sci. U.S.A.* **44**, 25-27.
- Scharrer, B., and Lochhead, M. S. (1950). Tumors in the invertebrates: A review. *Cancer Res.* **10**, 403-419.
- Smith, G. M. (1934). A mesenchymal tumor in an oyster (*Ostrea virginica*). *Am. J. Cancer* **22**, 838-841.
- Sparks, A. K. (1972). "Invertebrate Pathology: Noncommunicable Diseases," 387 pp. Academic Press, New York.
- Sparks, A. K. (1985). "Synopsis of Invertebrate Pathology Exclusive of Insects," 423 pp. Elsevier Amsterdam.
- Sparks, A. K., and Lightner, D. V. (1973). A tumorlike papilliform growth in the brown shrimp (*Penaeus aztecus*). *J. Invertebr. Pathol.* **22**, 203-212.
- Sparks, A. K., and Pauley, G. B. (1963). Studies in oyster pathology. Research in fisheries, 1962. *Univ. Wash., Coll. Fish., Contrib.* **147**, 51-53.
- Sparks, A. K., and Pauley, G. B. (1964). Studies in oyster pathology. Research in fisheries, 1963. *Univ. Wash., Coll. Fish., Contrib.* **166**, 59-60.
- Sparks, A. K., Pauley, G. B., Bates, R. R., and Sayce, C. S. (1964). A mesenchymal tumor in a Pacific oyster, *Crassostrea gigas* (Thunberg). *J. Insect Pathol.* **6**, 448-452.
- Sparks, A. K., Pauley, G. B., and Chew, K. K. (1969). A second mesenchymal tumor from a Pacific oyster (*Crassostrea gigas*). *Proc. Natl. Shellfish. Assoc.* **59**, 35-39.
- Steinhaus, E. A., and Martignoni, M. E. (1967). An abridged glossary of terms used in invertebrate pathology. *U.S. Dep. Agric., For. Serv. Pacific Northwest Forest and Range Experiment Station*, 22 pp.
- Taylor, R. L., and Smith, A. C. (1966). Polypoid and papillary lesions in the foot of the gaper clam, *Tresus nuttali*. *J. Invertebr. Pathol.* **8**, 264-266.
- Twomey, E., and Mulcahy, M. F. (1984). A proliferative disorder of possible haemic origin in the common cockle, *Cerastoderma edule*. *J. Invertebr. Pathol.* **44**, 109-111.
- Twomey, E., and Mulcahy, M. F. (1988). Transmission of a sarcoma in the cockle *Cerastoderma edule* (Bivalvia: Mollusca) using cell transplants. *Dev. Comp. Immunol.* **12**, 195-200.
- Wolf, P. H. (1969). Neoplastic growth in two Sydney rock oysters, *Crassostrea commercialis* (Iredale and Roughley). *Natl. Cancer Inst. Monogr.* **31**, 563-573.
- Wolf, P. H. (1976). Studies on the geographical distribution, etiology and transmission of integumentary epitheliomas in rock oysters from Australian estuaries. *Prog. Exp. Tumor Res.* **20**, 295-303.
- Yevich, P. P., and Barry, M. M. (1969). Ovarian tumors in the quahog *Mercenaria mercenaria*. *J. Invertebr. Pathol.* **14**, 266-267.
- Yevich, P. P., and Barszcz, C. A. (1976). Gonadal and hematopoietic neoplasms in *Mya arenaria*. *Mar. Fish. Rev.* **38**(10), 42-43.
- Yevich, P. P., and Barszcz, C. A. (1977). Neoplasia in soft-shell clams (*Mya arenaria*) collected from oil-impacted sites. *Ann. N.Y. Acad. Sci.* **298**, 409-426.

9

Genetic and Environmentally Induced Abnormalities

I. INTRODUCTION

The definition of “disease” used with reasonable consistency in this book is “any departure from normal structure or function of the animal.” Such a definition obviously includes many kinds of abnormalities, regardless of their origin. Etiology may be infectious or noninfectious, or a mixture of the two; and of the noninfectious diseases, causes may be genetic or environmental, or any mixture of the two. To keep some measure of order in this heterogeneous array of disorders, it might be good at the onset to list the most common causes of structural abnormalities in molluscs and in crustaceans. They include (but are certainly not limited to):

1. The effects of infectious diseases—especially parasitic diseases such as those caused by rhizocephalans, which result in gross modifications of host morphology as well as physiology
2. The effects of early genetic damage, to the egg, embryo, or larva, which modify subsequent development and may be lethal or sublethal, depending on their severity
3. The effects of abnormal environmental factors—particularly temperature, salinity, and oxygen—on sensitive developmental stages (included here would be abnormal chemical environments resulting from toxic algal blooms or pollution)
4. The effects of ectoparasites, commensals, or epibionts, which may modify shell growth in molluscs or inhibit molting in crustaceans
5. The effects of mechanical injuries, such as fracture of the shell of a mollusc or the exoskeleton of a crustacean

6. The effects of substrates (in the case of oysters and some other bivalves), in which the shape of the shell may be modified

Item 1 in this listing—effects of infectious diseases—has been considered in varying depth in Part I of this volume, which was concerned with a systematic treatment of infectious agents. This chapter deals with much of the remainder: noninfectious diseases (exclusive of tumors, which were considered in the preceding chapter). In accord with a general plan used throughout the volume, abnormalities in molluscs are considered first, then those of crustaceans.

II. MOLLUSCAN ABNORMALITIES

A number of abnormalities, other than those resulting from activities of pathogens and parasites, have been observed and reported in molluscs. Some of these seem to be of genetic origin, and others may be environmentally induced. Among the bivalves, shell and siphon abnormalities have been reported most frequently.⁸

The shell of a mollusc is secreted by the mantle and can be modified by abnormal mantle activity. Probably the best example of this was observed and reported by Merrill (1967). Extreme shell deformity in the sea scallop, *Placopecten magellanicus*, was produced by overgrowth of the growing edge by the colonial hydrozoan, *Hydractinia echinata*. Zooids of the coelenterate, armed with nematocysts, cause the mantle to retract. To resume growth, the scallop must successfully overgrow the hydroid colony; it does so by secreting an entirely new shell margin (Fig. 35). Sometimes the process of retraction and successful new growth is repeated several times, producing a grossly abnormal shell. Also, if the hydroid colony is confined to only one area of the shell, distortions in symmetry result, because growth is inhibited along only part of the shell margin.

One family of gastropods, the Pyramidellidae, contains members ectoparasitic on commercial marine bivalves—oysters, mussels, clams, and scallops—as well as on other marine invertebrates (Fretter and Graham, 1949). These very small snails feed on the body fluids of their hosts, and in so doing, produce morphological and physiological changes. Several species of the genus *Odostomia* parasitize oysters.

⁸A useful distinction can be made between two types of abnormal bivalve shells: anomalies, which are "deviations from a common type in excess of normal variations," and malformations, which are "harmful abnormalities that interfere with the functions of the organism" (Galtsoff, 1969).



Figure 35. Gross deformity of a scallop shell, caused by growth of the coelenterate *Hydractinia*.

Odostomia bisuturalis and *Odostomia eulimoides* cause severe damage to the oyster hosts. Loosanoff (1956) observed that damage to the mantle edge of *Crassostrea virginica* caused by activity of *O. bisuturalis* resulted in deformed shells (deeply cupped, with thickened margins). Cole and Hancock (1955) noted that *Ostrea edulis* parasitized by *O. eulimoides* also had irregular, thickened margins, often with multiple edges. These authors also felt that *Odostomia* parasitization interfered with the normal physiology of the oyster. Retraction of the mantle eventually led to atrophy of the adductor muscle and death. Another species, *Odostomia impressa*, has also been reported in American oysters (Hopkins, 1956a; Allen, 1958; Wells, 1959a).

The ectoparasitic pyramidellid snail, *Odostomia seminuda*, has been reported in bay scallops and calico scallops from the east coast of the United States (Adams, 1839; Hackney, 1944; Wells and Wells, 1961). Merrill and Boss (1964) found that the proboscis of the snail was able to pierce the mantle and the visceral mass of the host. Other members of

the genus reported to parasitize scallops are *Odostomia scalaris* from *Chlamys opercularis* in Denmark (Ankel and Møller-Christensen, 1963) and *O. eulimoides* from several genera of scallops.

Certain shell abnormalities in oysters and other bivalves may result from activities of boring animals. Outstanding in this respect are annelids of the genus *Polydora* and sponges of the genus *Cliona*. *Polydora* causes the formation of mud blisters on the inner shell of oysters in many parts of the world (Haswell, 1885; Leloup, 1937; Kavanagh, 1940; Lunz, 1940; Medcof, 1946; and others). The worms penetrate to the inner surface of the shell, where they are sealed off by a nacreous layer. If penetration occurs in the region of the adductor muscle, the oyster may be rendered more vulnerable to predators, such as crabs or starfish. Boring sponges have long been known as destructive pests of oysters. Giard (1881) reported that *Cliona* was known to oystermen of France in the form of "spice bread disease," which was claimed to destroy entire beds of oysters. Often, oyster shells are so completely riddled that they crumble with the slightest touch. An extensive literature on boring sponges exists (Giard, 1881; Old, 1941; Hopkins, 1956a,b, 1962; Wells, 1959b; and others).

Other than these shell malformations with known causes, abnormalities of unknown origin have been reported in several species of bivalves (Morse, 1923; Blake, 1929; Fisher, 1932; Clench, 1948; Medcof, 1949; Shuster, 1966; Logue, 1979). In one study European flat oysters, *Ostrea edulis*, being grown in Maine waters exhibited green conchiolinous deposits on the inner shell surfaces, concentrated at or near the area of attachment of the adductor muscle. The description of the abnormality is reminiscent of the fungus-induced *maladie du pied* of the European oyster, although no etiological agent was identified.

Next to shell malformations, abnormal siphons are the most common gross abnormality seen in bivalves. Supernumerary siphons, either functional or nonfunctional, were reported in quahaugs, *Mercenaria mercenaria*, and soft-shell clams, *Mya arenaria* by Potter and Kuff (1967) and by Tubiash *et al.* (1968). Causes were unknown, but developmental aberrations or abnormal regeneration after injury was suggested. Similarly, bifurcation of the foot of clams has been seen occasionally (Pelseneer, 1923; Atkins, 1931), as has bifurcation of the adductor muscle of oysters (Gunter, 1958; Pauley and Sayce, 1967).

Two categories of concretions—pearls and "kidney stones"—occur in bivalves and may fit the designation of abnormalities. Pearl formation is, of course, the subject of a massive literature, some of it dating back more than a century (Garner, 1873). Fortunately, much of the information has been reviewed by Cheng (1967) and by Lauckner (1983), who

concluded that, while larval trematode invasion is often involved in initiating the process, other agents, such as sporozoan spores, cestode larvae, and even foreign objects such as sand grains, may also induce pearl formation. Pearl production in mussels, *Mytilus edulis*, has been studied extensively, principally because of negative economic effects (see, for example, Scattergood and Taylor, 1949; Lutz and Hidu, 1978).

Information about excretory system concretions of calcium phosphate in bivalves is of more recent origin (Potts, 1967; Doyle *et al.*, 1978; Gold *et al.*, 1982). Clams (*M. mercenaria*) and scallops (*Argopecten irradians*) from the U.S. East Coast develop such concretions, which may occlude the entire kidney, and a relationship with polluted or abnormal environments has been proposed, based on the successful experimental induction of the deposits in the wedge shell, *Donax trunculus* (Mauri and Orlando, 1982), and on the finding that concretions in clams from polluted sites were more numerous and larger than those in clams from reference sites.

Other (and rarer) abnormalities have been reported from bivalves. A "black oyster" was reported by Pauley and Sayce (1967); the interior of the shell was discolored and the entire cross section of the animal was black, except for the adductor muscle.

As is true with fish, abnormalities have been observed in molluscs in aquaculture facilities, where environments are marginal at best, and where opportunities for visual inspection are abundant. Effects of infectious diseases are of the greatest concern, since they retard growth and often result in mortalities, but, occasionally, abnormalities with probable noninfectious etiologies are seen in captive populations. An example of abnormal bivalve shell development resulting from mantle pathology was reported by Leibovitz *et al.* (1976). Shell deformations, in the form of asymmetrical elevations and ridges, were seen in hatchery-reared clams (*M. mercenaria*), coincident with a hyperplastic condition of the mantle. An infectious disease was suspected but not demonstrated.

III. CRUSTACEAN ABNORMALITIES

Pigmentation and structural abnormalities are well known in Crustacea. Particularly numerous are reports of partial albinism and the absence of certain pigments in crabs and lobsters. White American lobsters, *Homarus americanus*, were reported by Templeman (1948). He also noted a bicolored condition seen earlier by Herrick (1895, 1909). A similar bicolored condition has been seen repeatedly in the European lobster, *Homarus gammarus* (Schaanning, 1929; Dexter, 1959). Lobsters with this

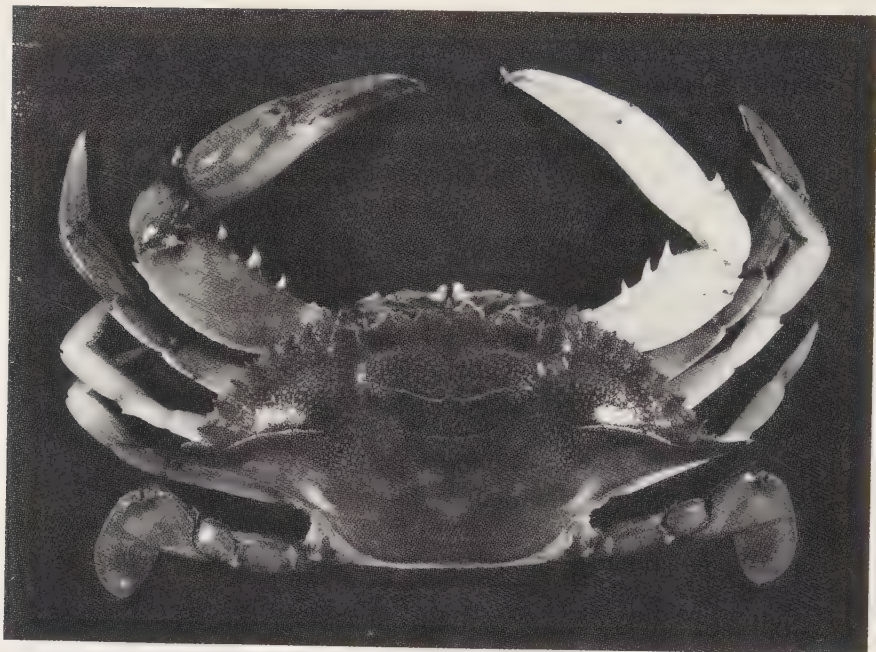


Figure 36. Partial albinism in a blue crab, *Callinectes sapidus*.

condition have an exact dividing line in the center of the cephalothorax and the abdomen, with normal pigmentation on one side and red, blue, or white color on the other. Some individuals had other combinations of colors, but the exact bilateral dividing line suggests modification in pigment-producing genes in the earliest division of the egg. Still other individuals have abnormal coloration confined to certain appendages or certain parts of the body. Chace and Moore (1959) even found a bi-colored lobster which was also a gynandromorph, again suggesting genetic change in the first egg division. Studies of lobsters which were all red or all blue have indicated some details of the genetic basis of exoskeletal pigmentation. Crosses of color variants have been made, and use of the offspring as subpopulation markers has been proposed (Hughes, 1968).

Color variations in blue crabs, *Callinectes sapidus*, have also been described (Rathbun, 1930; Newcombe, 1945; Haefner, 1961; Sims and Joyce, 1965). Partial albinism, especially in the appendages, is often seen (Fig. 36). Complete albinism is less common, but several examples have been reported.

Shrimp also have color variations. One of the most common is a

generalized condition of the flesh and the exoskeleton that gives a "golden" appearance to the affected individual. The condition is thought to have a genetic basis (Overstreet, 1973; Johnson, 1978).

Exoskeletal anomalies are also common in the larger Crustacea. Abnormal chelipeds in lobsters and in crabs have received particular attention in the literature. Herrick (1895), Emmel (1907), and Templeman (1948) all observed lobsters with two crusher claws, and more rarely two biting claws. Cole (1910) found a double extra claw on the cheliped of a lobster, and he was one of many who have noted extra processes on the claws of crabs (Faxon, 1881; Leavitt, 1909; Verrill, 1908). The spectrum of claw anomalies in decapods has been well illustrated by Shuster *et al.* (1963). These investigators pointed out that such anomalies occur most frequently in the chelipeds, which have the greatest capacity for regeneration, and that they probably are most often produced by injury while the exoskeleton is soft. The authors also pointed out, however, that some abnormalities persisted through molting.

An exoskeletal abnormality with suspected environmental involvement has been reported in the shrimp, *Penaeus stylirostris*, grown in raceway culture near the Gulf of California (Lightner *et al.*, 1984). Labeled "blunted head syndrome," the abnormality, with accompanying mortalities, occurred in 10–50% of the individuals in raceway populations. Affected shrimp develop blunted head appendages (e.g., antennae, antennules, rostrum, antennal blades), due to erosion. The abnormality has been linked circumstantially with red tide outbreaks in adjacent waters, although overcrowding may be a contributing factor.

This example of blunted head syndrome highlights the point that a number of abnormalities of cultured animals do not have an apparent infectious etiology. As aquaculture production of animals such as oysters and shrimp expands, more and more of these disease conditions are observed and reported. A recent summary of penaeid shrimp diseases listed 28 disease conditions, of which one third were of noninfectious etiology—related to nutritional or environmental deficiencies (Lightner, 1988).

IV. CONCLUSIONS

Perusal of this chapter leads quickly to the conclusion that most of these abnormalities are not of great importance to survival of the affected animal. Some may be of scientific interest (as, for example, illustrations of the extreme plasticity of molluscan shell growth or the genetic basis of pigmentation in crustaceans), but they seem to play little part in population regulation—unlike the infectious diseases.

Of course, what is observed and reported is that small part of the population with sublethal abnormalities that permit the animal to survive to juvenile or adult stages; all of the earlier life stages with more serious defects have long since been removed from the population.

A further qualification is that genetic or environmentally induced abnormalities may occur with high prevalences in aquaculture populations. Some examples of this have been reported (e.g., skeletal deformities and color variants in fish, shell deformities in clams, blunted heads in shrimp). Such defects may affect marketability as well as survival.

Recent interest in pollution effects on marine populations and in the use of abnormalities of marine animals as indicators of environmental degradation has resulted in examination of molluscs and crustaceans from these perspectives. The possible relationships of infectious diseases of fish to pollution have received most of the attention, but there is some evidence for increased prevalences of abnormalities of noninfectious origin in invertebrates—especially crustaceans—from degraded habitats.

REFERENCES

- Adams, C. B. (1839). Observations on some species of the marine shells of Massachusetts, with descriptions of five new species. *Boston J. Nat. Hist.* **2**, 262–289.
- Allen, J. F. (1958). Feeding habits of two species of *Odostomia*. *Nautilus* **72**, 11–15.
- Ankel, F., and Møller-Christensen, A. (1963). Non-specificity in host selection by *Odostomia scalaris* MacGillivray. *Vidensk. Meddr. Dansk Naturh. Foren.* **125**, 321–325.
- Atkins, D. (1931). Note on some abnormalities of labial palps and foot of *Mytilus edulis*. *J. Mar. Biol. Assoc. U.K.* **17**(1930/31), 545–550.
- Blake, J. H. (1929). An abnormal clam. *Nautilus* **38**, 89–90.
- Chace, F. A., Jr., and Moore, G. M. (1959). A bicolored gynandromorph of the lobster, *Homarus americanus*. *Biol. Bull. (Woods Hole, Mass.)* **116**, 226–231.
- Cheng, T. C. (1967). Marine molluscs as hosts for symbioses. With a review of known parasites of commercially important species. *Adv. Mar. Biol.* **5**, 1–424.
- Clench, W. J. (1948). A remarkable malformed specimen of *Venus campechiensis* Gmelin. *Rev. Soc. Malacol.* **6**, 10.
- Cole, L. J. (1910). Description of an abnormal lobster cheliped. *Biol. Bull. (Woods Hole, Mass.)* **18**, 252–268.
- Cole, H. A., and Hancock, D. A. (1955). *Odostomia* as a pest of oysters and mussels. *J. Mar. Biol. Assoc. U.K.* **34**, 25–31.
- Dexter, R. W. (1959). Blue lobsters. *Estuarine Bull.* **4**(2), 6–7.
- Doyle, L. J., Blake, N. J., Woo, C. C., and Yevich, P. P. (1978). Recent biogenic phosphorite: Concretions in mollusk kidneys. *Science* **199**, 1431–1433.
- Emmel, V. E. (1907). Regenerated and abnormal appendages in the lobster. *Annu. Rep. R. I. Comm. Inland Fish.*, 37th pp. 99–152.
- Faxon, W. (1881). On some crustacean deformities. *Bull. Mus. Comp. Zool.* **8**(13), 257–274.
- Fisher, N. (1932). Malformation in *Mya arenaria* L. *J. Conchol.* **19**, 270.

- Fretter, V., and Graham, A. (1949). The structure and mode of life of the Pyramidellidae, parasitic opisthobranchs. *J. Mar. Biol. Assoc. U.K.* **28**, 493–532.
- Galtsoff, P. S. (1969). Anomalies and malformations in the shells of *Crassostrea virginica*. *Natl. Cancer Inst. Monogr.* **31**, 575–580.
- Garner, R. (1873). On the formation of British pearls and their possible improvement. *J. Linn. Soc. London, Zool.* **11**, 426–428.
- Giard, A. (1881). Fragments biologiques. II. Deux ennemis de l'ostréiculture. *Annu. Bull. Sci. Dep. Nord.*, 4th pp. 70–73.
- Gold, K., Capriulo, G., and Keeling, K. (1982). Variability in the calcium phosphate concretion load in the kidney of *Mercenaria mercenaria*. *Mar. Ecol. Prog. Ser.* **10**, 97–99.
- Gunter, G. (1958). An abnormal Virginia oyster with a bifurcated muscle. *Proc. Natl. Shellfish. Assoc.* **48**, 152–153.
- Hackney, A. G. (1944). List of Mollusca from around Beaufort, N. Carolina, with notes on *Tethys*. *Nautilus* **58**, 56–64.
- Haefner, P. A., Jr. (1961). A blue blue crab. *Estuarine Bull.* **6**, (3/4), 3–5.
- Haswell, W. A. (1885). On a destructive parasite of the rock oyster. *Proc. Linn. Soc. N.S.W.* **10**, 273–275.
- Herrick, F. H. (1895). "The American Lobster: A Study of Its Habits and Development," 252 pp. Government Printing Office, Washington, D.C.
- Herrick, F. H. (1909). Natural history of the American lobster. *Bull. Bur. Fish.* **29**, 149–408.
- Hopkins, S. H. (1956a). *Odostomia impressa* parasitizing southern oysters. *Science* **124**, 628–629.
- Hopkins, S. H. (1956b). Notes on the boring sponges in Gulf coast estuaries and their relation to salinity. *Bull. Mar. Sci. Gulf Caribb.* **6**, 44–58.
- Hopkins, S. H. (1962). Distribution of species of *Cliona* (boring sponge) on the eastern shore of Virginia in relation to salinity. *Chesapeake Sci.* **6**, 44–58.
- Hughes, J. T. (1968). Red lobsters signal breakthrough. *Natl. Fisherman* **48**(12), 13A, 22A.
- Johnson, S. K. (1978). Handbook of shrimp diseases. *Tex. A&M Univ. Sea Grant Program [Rep.] TAMU-SG-75-603*, 23 pp.
- Kavanagh, L. D. (1940). Mud blisters in Japanese oysters imported to Louisiana. *Louisiana Conserv. Rev.* **Autumn**, 31–34.
- Lauckner, G. (1983). Diseases of Mollusca: Bivalvia. In "Diseases of Marine Animals" (O. Kinne, ed.), Vol. 2, Biol. Anst. Helgol., Hamburg, FRG.
- Leavitt, R. G. (1909). An interesting crab's claw. *Guide Nature* **3**(3), 89–91.
- Leibovitz, L., Meyers, T. R., and Frey, M. (1976). A shell deforming disease of hard clams (*Mercenaria mercenaria*). *Proc. Int. Colloq. Invertebr. Pathol.*, 1st pp. 386–387.
- Leloup, E. (1937). Contributions à l'étude de la faune Belge. VIII. Les dégâts causés par le ver polychete *Polydora ciliata* (Johnston) dans les coquilles des bigorneaux et des huîtres. *Bull. Mus. R. Hist. Nat. Belg.* **13**, 1–4.
- Lightner, D. V. (1988). Diseases of cultured penaeid shrimp and prawns. In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann and D. V. Lightner, eds.), pp. 8–133. Elsevier Amsterdam.
- Lightner, D. V., Redman, R. M., Danald, D. A., Williams, R. R., and Perez, L. A. (1984). Major diseases encountered in controlled environment culture of penaeid shrimp at Puerto Penasco, Sonora, Mexico. *NOAA Tech. Rep.*, NMFS Circ. **16**, 25–33.
- Logue, M. D. (1979). Abnormalities in the shell of the Maine-grown European oyster (*Ostrea edulis*). *Proc. Natl. Shellfish. Assoc.* **69**, 196–197.
- Loosanoff, V. L. (1956). Two obscure oyster enemies in New England waters. *Science* **123**, 1119–1120.
- Lunz, G. R. (1940). The annelid worm, *Polydora*, as an oyster pest. *Science* **92**, 310.

- Lutz, R. A., and Hidu, H. (1978). Some observations on the occurrence of pearls in the blue mussel, *Mytilus edulis* L. *Proc. Natl. Shellfish. Assoc.* **68**, 17–37.
- Mauri, M., and Orlando, E. (1982). Experimental study on renal concretions in the wedge shell *Donax trunculus* L. *J. Exp. Mar. Biol. Ecol.* **63**, 47–57.
- Medcof, J. C. (1946). The mud-blister worm, *Polydora*, in Canadian oysters. *J. Fish. Res. Board Can.* **6**, 498–565.
- Medcof, J. C. (1949). Dark-meat and the shell disease of scallops. *Fish. Res. Board Can., Prog. Rep. Atl. Coast Stn.* **45**, 3–6.
- Merrill, A. S. (1967). Shell deformity of mollusks attributable to the hydroid, *Hydractinia echinata*. *U.S. Fish Wildl. Serv., Fish. Bull.* **66**, 273–279.
- Merrill, A. S., and Boss, K. J. (1964). Reactions of hosts to proboscis penetration by *Odostomia seminuda* (Pyramidellidae). *Nautilus* **78**, 42–45.
- Morse, E. S. (1923). An abnormal shell of *Mya arenaria*. *Nautilus* **36**, 28–30.
- Newcombe, C. L. (1945). The biology and conservation of the blue crab *Callinectes sapidus* Rathbun. *Va. Fish. Lab., Ed. Ser.* **4**, 1–39.
- Old, M. C. (1941). The taxonomy and distribution of the boring sponges (Clinoidae) along the Atlantic coast of North America. *Chesapeake Biol. Lab.* **44**, 1–30.
- Overstreet, R. M. (1973). Parasites of some penaeid shrimps with emphasis on reared hosts. *Aquaculture* **2**, 105–140.
- Pauley, G. B., and Sayce, C. S. (1967). Description of some abnormal oysters (*Crassostrea gigas*) from Willapa Bay, Washington. *Northwest Sci.* **41**, 155–159.
- Pelseeneer, P. (1923). Variations dans les mollusques. *Ann. Soc. R. Zool. Belg.* **54**, 68–78.
- Potter, M., and Kuff, E. (1967). A developmental anomaly of the siphon of the soft-shell clam, *Mya arenaria*, from Chesapeake Bay. In "Activities Report of the Registry of Tumors in Lower Animals for the Period September 1, 1966, to March 31, 1967" (J. C. Harshbarger, ed.), p. 11. Smithsonian Inst., Washington, D.C.
- Potts, W. T. W. (1967). Excretion in the mollusks. *Biol. Rev.* **41**, 1–41.
- Rathbun, M. J. (1930). The cancrroid crabs of America of the families Euryalidae, Portunidae, Atelecyclidae, Cancridae and Xanthidae. *Bull. U.S. Natl. Mus.* **152**, 1–609.
- Scattergood, L. W., and Taylor, C. C. (1949). The mussel resources of the North Atlantic region. Part II. Observations on the biology and the methods of collecting and processing the mussel. *Commer. Fish. Rev.* **11**(10), 11–23.
- Schaanning, H. T. L. (1929). Ein eiendommelig varietet av hummer (*Homarus vulgaris*). *Stavanger Mus. Aarsh.* **1925–28**(5), 1–3.
- Shuster, C. N., Jr. (1966). A uniquely shaped quahog. *Maritimes* **10**, 14.
- Shuster, C. N., Jr., Ulmer, D. H. B., Jr., and Van Engel, W. A. (1963). A commentary on claw deformities in the blue crab. *Estuarine Bull.* **7**, (2/3), 15–23.
- Sims, H. W., and Joyce, E. A. (1965). Partial albinism in a blue crab. *Q. J. Fla. Acad. Sci.* **28**, 373–374.
- Templeman, W. (1948). Abnormalities in lobsters. *Bull. Newfoundland Govt. Lab.* **18**, 3–8.
- Tubiash, H. S., Shuster, C. N., Jr., and Couch, J. A. (1968). Anomalous siphons in two species of bivalve mollusks. *Nautilus* **81**, 120–125.
- Verrill, A. E. (1908). Decapod Crustacea of Bermuda, I. Brachyura and Anomura. *Trans. Conn. Acad. Arts Sci.* **13**, 299–474.
- Wells, H. W. (1959a). Notes on *Odostomia impressa* (Say). *Nautilus* **72**, 140–144.
- Wells, H. W. (1959b). Boring sponges (Clionidae) of Newport River, North Carolina. *J. Elisha Mitchell Sci. Soc.* **75**, 168–173.
- Wells, H. W., and Wells, M. J. (1961). Three species of *Odostomia* from North Carolina, with description of new species. *Nautilus* **74**, 149–157.

Part IV

Responses of Shellfish to Pathogens

Survival in coastal/estuarine habitats requires that shellfish possess an armamentarium of defenses—external as well as internal—against invasion by infectious agents of all types, from viruses to metazoan parasites. The essential ingredients in the internal defense network are both cellular and humoral, acting in a totally integrated system to diminish the effects of, and to eliminate, invading agents.

Phagocytosis by fixed and mobile cells is the principal cellular defense of shellfish, with lysosomal enzymes within phagocytic vacuoles and in the hemolymph assuming a critical role in killing microorganisms. Focal tissue abscesses and shell pustules represent terminal activities by molluscs to isolate and eliminate pathogens; rupture of abscesses or diapedesis of phagocytes through the mantle epithelium is often accompanied by the formation of shell pustules containing dead phagocytes. A counterpart sequence in crustaceans would be the formation of “black gills,” with aggregates of phagocytes, melanin deposition, and blockage of terminal portions of the gill filaments—followed by their subsequent loss at molting. Accessory cellular responses in shellfish include hemocytosis (aggregation of hemocytes), encapsulation (of invaders too large to be phagocytized), and, in the molluscs, nacrezation (secretion by the mantle of nacre around foreign bodies).

Humoral defenses of molluscan shellfish consist of preexisting and, in some instances, induced lysins and agglutinins, augmented by an array of lysosomal enzymes in hemocytes and hemolymph. Some equivocal evidence exists for increased antibacterial activity in animals exposed previously to bacterial antigens, and for heightened bacteriophage clearance after repeated challenges. Humoral defenses in crustaceans are also characterized by the presence of naturally occurring bactericidins, lysins, and agglutinins which have, in some studies, been experimentally enhanced by previous exposure to antigens, but which, like molluscan humoral factors, are of much lower specificity than vertebrate immunoglobulins.

This combination of cellular responses (hemocytosis and phagocytosis, with a capacity for phagocyte activation) and humoral responses (naturally occurring and induced antimicrobial activity of the hemolymph) seems to be the core of the internal defense system of molluscan and crustacean shellfish. In Chapter 10 the system is dissected into its component parts, and then some classic examples of interactions of host and pathogen will be discussed (i.e., the protozoan *Haplosporidium nelsoni* in oysters and the bacterium *Aerococcus viridans* in lobsters). The chapter, in retrospect, seems very detailed, but maybe this is sensible, since mechanisms of resistance to disease are, of course, critical to survival.

Mechanisms of Internal Defense

I. INTRODUCTION

The literature on internal defense mechanisms of invertebrates is voluminous. General reviews are abundant, and some of the earlier ones are still useful (Huff, 1940; Hirsch, 1959; Stauber, 1961; Tripp, 1963; Bang, 1967a,b; Cushing, 1967; Feng, 1967). More recent reviews include those by Bang (1970, 1973), Sindermann (1971), Schapiro (1975), Tripp (1975), Cheng (1981), Warr (1981), Bayne (1983), Durliat (1985), Fries (1985), Ratcliffe *et al.* (1985), Söderhäll (1986), Johnson (1987), and Sminia and van der Knapp (1987). Several recent books contain material on invertebrate internal defenses (Maramarosch and Shope, 1975; Cooper, 1976; Manning and Turner, 1976; Solomon and Horton, 1977; Brehélin, 1986).

Some of the terms used in discussing the internal defenses of animals include: "susceptibility," "resistance," "immunity," "innate resistance," and "acquired resistance." Often, the precise limits of such terms are not clearly defined. Schneider (1951), Read (1958), and Stauber (1961), for example, pointed out that "susceptibility" and "resistance" should be considered as separate biological entities. "*Susceptibility*" should be limited to the *degree of vulnerability* of an animal to penetration by a pathogen and to the successful establishment of the pathogen. Susceptibility has been further defined as "a physiological state of the host in which the parasite (pathogen) is supplied with its life needs," and insusceptibility is "the state in which these life needs are not satisfied—neither state involving a host response" (Read, 1958). "*Resistance*," on the other hand, should be restricted to the *responses* of an animal to invasion by parasites or pathogens, and may be defined as "those alterations of the physiological state of the host which represent a response to previous or present experience with the parasite (pathogen) or a chemically related entity" (Read, 1958).

Resistance, when properly restricted to responses of the host to

parasite invasion, is usually divided into two categories: innate (natural) and acquired. Innate resistance depends on those responses of the host that appear upon first contact with a pathogen. It is usually a species or racial characteristic, but it may vary individually. Acquired resistance, as succinctly described by Stauber (1961), is host response which develops after initial exposure to an invading organism or its metabolites; if the host survives this contact, acquired resistance (immunity) may persist for varying periods once it has developed. It may be characterized by enhanced response to the invasive organism upon subsequent contact with it, and—in the vertebrates only—it is usually associated with the presence of specific antibodies (Stauber, 1961).

The mechanisms of resistance of the animal, whether innate or acquired, may be cellular or humoral. Cellular responses of invertebrates include the following: (1) phagocytosis and the digestion of foreign particles, or transport of the particles across an epithelial surface if they are indigestible (diapedesis); (2) hemocytosis and hemocytic infiltration—the mobilization of phagocytic and other cells in the hemolymph and their migration toward sources of irritation; (3) thrombosis—the formation of cellular or extracellular clots to close gaps in the vascular system and immobilize invading microorganisms; (4) encapsulation—a response to the presence of a large amount of foreign material or to certain types of indigestible material, often characterized by envelopment by concentric layers of fibroblastlike cells. Humoral responses of invertebrates are actually special cases of cellular responses, in which “Cellular secretion, fragmentation, or biochemical alteration confers bacteriostatic, lytic, or other properties on body fluid” (Stauber, 1961). If these properties are present before introduction of microorganisms, then they should properly be considered insusceptibility factors rather than factors of resistance, as was pointed out by Stauber. Some invertebrate body fluids do have striking bacteriolytic properties, as well as agglutinating activity which may facilitate phagocytosis and encapsulation. It is usually very difficult, however, to determine whether or not such properties result from previous experience with a pathogen. Recent but incomplete evidence for augmentation of bactericidal activity in the body fluids of a few invertebrates (considered later in the chapter) suggests that mechanisms of resistance are operative, possibly in addition to insusceptibility factors (Fig. 37).

This chapter is concerned with internal defense mechanisms of marine molluscs and crustaceans, recognizing that additional data exist for other phyla, and that the “nonvertebrates” constitute an assemblage of evolutionarily very diverse animals with possibly very different ways of responding to the invasion of pathogens. An attempt has been made to

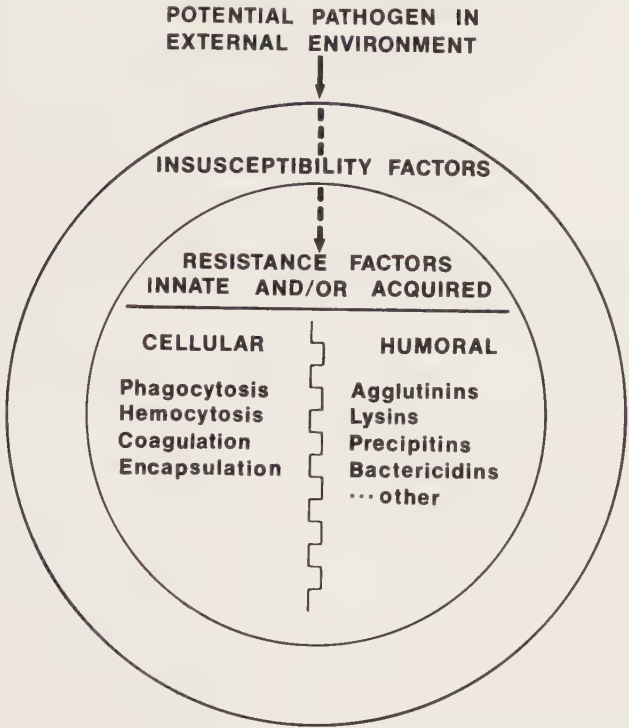


Figure 37. Mechanisms of internal defense in invertebrates.

trace the historical development of knowledge in each of the following subsections, since the internal defense mechanisms of invertebrates have long fascinated (and baffled) investigators.

II. MECHANISMS OF INTERNAL DEFENSE IN MOLLUSCS

Responses of molluscs to infection involve cellular and humoral components, just as they do in vertebrates. Phagocytosis and hemocytic infiltration are the primary cellular responses, while an array of hemolymph factors—such as, lysins, agglutinins, and enzymes—normally present or inducible, constitutes the principal humoral defenses. It has been aptly pointed out, though, that all internal defenses are ultimately cellular, since humoral factors are of cellular origin. To clarify and reinforce this point, Kinne (1980, p. 46) distinguished between “whole cell” mechanisms (phagocytes and other hemocytes) and “cell product” mechanisms (secretions or cell fragments released into body fluids). These

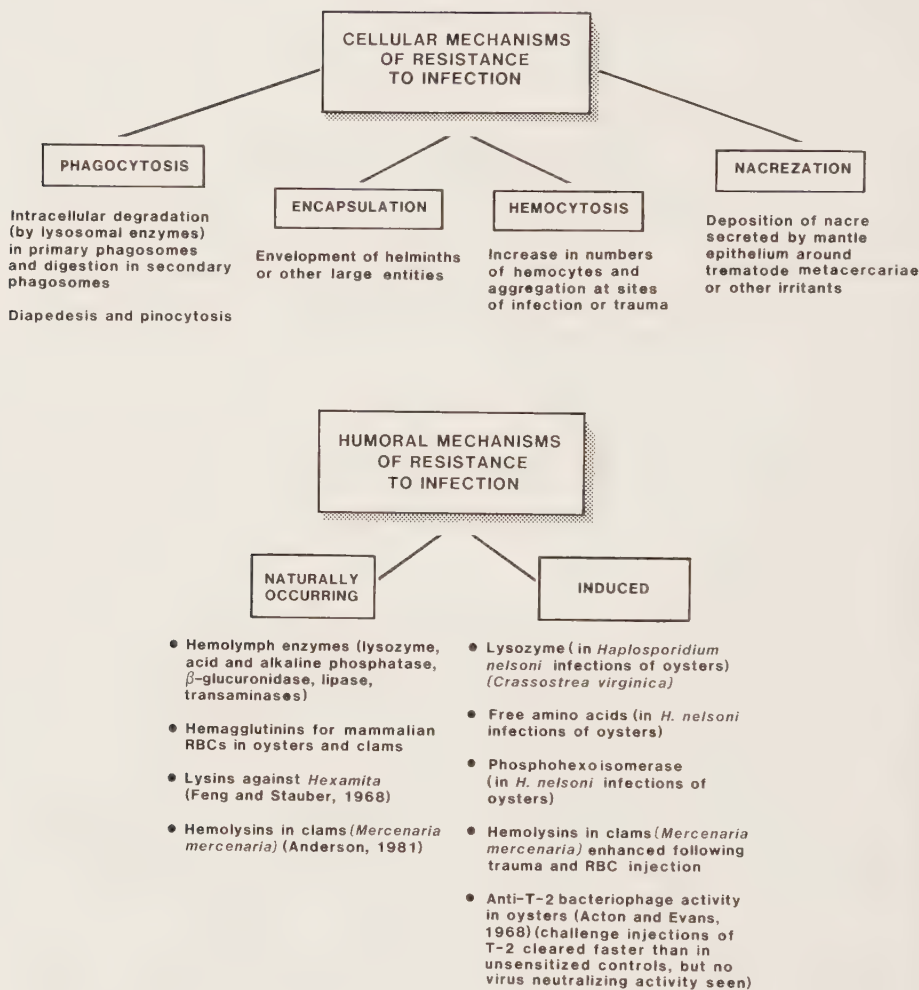


Figure 38. Cellular and humoral mechanisms of internal defense in molluscs.

mechanisms are cooperative and integrated to the point at which differences are almost indistinguishable. It is possible, though, to separate them artificially for discussion purposes, as has been done in Fig. 38, keeping in mind the mutual activity that is thus being ignored and also recognizing that some defensive properties are already present in the hemolymph of normal animals, while others are inducible.

This section on internal defenses of molluscs follows customary practice by reviewing first the cellular (whole cell) mechanisms, and then the humoral (cell product) mechanisms. After that, a case history of

resistance of oysters to a specific disease is considered, in an attempt to relate events at the cellular/biochemical level to the disease process in natural populations.

A. Cellular Mechanisms of Defense

Cellular (whole cell) mechanisms of defense in bivalve molluscs have been described and categorized (Stauber, 1961; Cheng, 1981). Four kinds of defenses exist: phagocytosis, encapsulation, hemocytosis, and nacre-zation.

Phagocytosis, the uptake of foreign material (including pathogens) by host hemocytes, removing them from contact with tissues (Cheng, 1981), is the dominant cellular response in bivalve molluscs. The intracellular fate of ingested particles is varied: The digestible ones are degraded within the phagosomes of hemocytes; indigestible ones are stored within the cells or physically removed by migration of cells out of the host (Stauber, 1950; Tripp, 1960; Cheng, 1967; Feng, 1967). Additionally, some microorganisms may remain unchanged indefinitely within the phagocyte, or may multiply there, eventually killing the host cell.

All types of hemocytes of bivalves can be phagocytic, but granular cells play a most active role (Foley and Cheng, 1975). The granules in the cytoplasm of such cells are lysosomes, serving as storage areas for acid hydrolases (Yoshino and Cheng, 1976). Enzymatic digestion within the phagosome is followed by diffusion of nutrients into the cytoplasm. Lysosomal enzymes may also be released from granular hemocytes into the hemolymph, where antimicrobial activity may be initiated (Cheng, 1981).

Several parasites and pathogens of molluscs have been reported as being phagocytized: the protozoan *Perkinsus marinus* by Mackin (1951), the protozoan *Haplosporidium nelsoni* by Farley (1968), and the flagellate *Hexamita nelsoni* by Mackin *et al.* (1952). One of the most enlightening studies of phagocytosis in molluscs was that by Bang (1961), who used *in vitro* methods with oyster hemocytes and bacteria. Bang found that bacteria adhered to the hemocyte before phagocytosis, in part because of entanglement of bacterial flagella by pseudopodia. He also found that certain bacteria were not phagocytized.

Encapsulation is the molluscan response of choice to invasion by a parasite or other foreign body too large to be phagocytized (Cheng and Rifkin, 1970). The process is variable, in terms of the kinds of host cells that participate; some are hemocytes, some are connective tissue cells, and some are muscle cells. Encapsulation is the principal molluscan

cellular reaction to helminth parasites, and it is especially pronounced if parasites are in abnormal hosts or abnormal locations or if the parasites are moribund or dead. Encapsulation eventually results in an envelope of concentric layers of cells around the invader, with intensity of response probably a function of the evolutionary association of parasite and host. The most intense response in bivalves is seen to larval cestodes and trematode metacercariae; disintegration of the parasite is followed by disintegration of the capsule and resorption of cellular debris (Cheng and Rifkin, 1970). It should be noted, though, that encapsulation does not necessarily lead to destruction of the parasite—unless extensive hemocyte and brown cell infiltration occurs. The hemocytic response and capsule formation are believed to be mutually exclusive processes (Cheng and Rifkin, 1970).

Encapsulation is a response to infections by such pathogens as the protozoan *P. marinus* and to invasion by larval cestodes and certain larval trematodes. Abscesses that are formed, particularly in the mantle, have outer margins of hemocytes and fibrous connective tissue. Eventually, the entire lesion may be walled off by an epithelial layer. Encapsulation has been demonstrated in snails penetrated by miracidia of trematodes abnormal to the species; the reaction occurred within 24 hours.

Hemocytosis, an increase in the number of hemocytes, is an obvious prelude to phagocytosis or encapsulation (Cheng and Rifkin, 1970). Aggregation of hemocytes is a typical response of bivalves to parasitization or microbial infection, although it is still uncertain whether the cells are newly formed in some hematopoietic center or whether they are preexisting cells released from other tissues (Cheng, 1981).

Knowledge about the value of hemocytic infiltration (inflammation) as an internal defense against infection is still incomplete. In the vertebrates inflammation involves a sequence of phenomena including changes in the permeability of blood vessels, leakage of blood fluids into tissues, adherence of leukocytes to blood vessel walls, and migration of leukocytes into tissues around the area of foreign invasion or injury. Counterparts of these phenomena exist in invertebrates, particularly for the adherence of hemocytes to blood vessel walls and migration into invaded or injured tissues. This behavior of hemocytes was effectively demonstrated in the American oyster by Bang (1961) as part of a very lucid explanation of internal defense processes in this species.

The various phases of hemocyte infiltration of tissues can be effectively demonstrated in oysters infected by *Haplosporidium nelsoni*. Infected oysters exhibit edema and cell infiltration, with small lymphocytelike hemocytes. Proportions of cell types shift toward greater relative numbers of these lymphocytelike cells—small, with large nu-

clei—which may or may not differentiate into phagocytes. Large hemocytes frequently ingest the parasites, but do not seem to kill them, and may only spread them systemically in the host.

The involvement of hemocytes in clot formation or thrombosis is complex and deserves particular attention, since either cellular or extracellular clots may be formed. Bang (1961) was able to demonstrate extensive intravascular cellular clots in oysters, caused by injecting an extract of ground gill tissue—an injury response. The clots adhered to the blood vessel wall, producing stasis of blood, and were resolved only very slowly. The presence of an extracellular gel or clot in oysters seems to inhibit bacterial motion and may thus render microorganisms more susceptible to phagocytosis. The origin of components or activators of the clotting process from hemocytes, many of which are granular, is obviously possible.

Nacrezation is a defense mechanism of molluscs in which nacre secreted by the mantle epithelium is deposited around parasites or foreign bodies which invade and irritate the mantle region. It is a process distinct from encapsulation, and hemocytes are not involved. Pearl formation is an outcome of nacrezation; induction may be by invasion of certain trematode metacercariae, by sand grains, or by artificial implants of mantle epithelium in subepithelial tissues (Tsujii, 1960; Cheng, 1967). The presence of dead helminth larvae or of other irritants in the tissues, or between the shell and mantle, will stimulate secretion and deposition of nacreous layers about the foreign material, often incorporating the material in blisters on the inner surface of the shell. A similar process occurs when shell-invading annelids, *Polydora*, penetrate through the inner shell surface. Farley (1968) described the process of recovery of oysters from *Haplosporidium nelsoni* infections, which included the walling off in shell pustules of phagocytized pathogens. This process, considered the terminal stage in removal of moribund pathogens, followed localization of phagocytized organisms in lesions or pustules in the mantle epithelium.

B. Humoral Mechanisms of Defense

Natural antibodylike substances—agglutinins, lysins, and various other antimicrobial factors—have been demonstrated in the body fluids of many invertebrates. Evidence for a wide range of natural lectinlike agglutinins in molluscs was summarized by Feng (1967). Hemagglutinins specific for human A antigen were reported by Johnson (1964) in the butter clam, *Saxidomus giganteus*. Oyster blood contains agglutinins for bird and mammal erythrocytes. Tripp (1966) reported oyster hemag-

glutinin to be heat labile, with an opsonic effect on the phagocytosis of rabbit erythrocytes by oyster hemocytes *in vitro*.

Specific adaptive immune responses, characterized by the production of immunoglobulins, have not been demonstrated in molluscs (or in other invertebrates), but other humoral (cell product) factors that are protective against certain disease agents *have* been demonstrated, and some limited evidence exists for short-term enhancement of this protective function by prior exposure to antigenic material. Thus, in one study naturally occurring hemolysins were found in hemocytes, hemolymph, and shell liquor of hard clams, *Mercenaria mercenaria*—and induction of heightened hemolytic activity was achieved by trauma and injection of blood cells. The response persisted for 3 days (Anderson, 1981).

The presence of lysins and agglutinins in molluscan hemolymph has been demonstrated frequently, and these molecules undoubtedly play important roles in the response to invasion by microorganisms. Agglutinins have been shown to act as opsonins, enhancing phagocyte activity (Arimoto and Tripp, 1977; Renwrantz *et al.*, 1981; van der Knaap *et al.*, 1982; Renwrantz and Stahmer, 1983; Leippe and Renwrantz, 1988). The agglutinin/opsonin is synthesized by hemocytes and occurs on the cell surface as receptors for foreign antigens (van der Knaap *et al.*, 1981a,b). Additionally, bivalve hemocytes have been shown to produce hydrogen peroxide in both resting and stimulated states *in vitro*, extending the list of nonspecific defense factors against invading microorganisms (Nakamura *et al.*, 1985). Also, cytotoxicity of molluscan hemocytes, similar to that of the "killer cell" function described for mammalian hemocytes (Sanderson, 1981; Kimber, 1985; Marx, 1986), has recently been reported in mussels and clams (Wittke and Renwrantz, 1984; Yoshino and Tuan, 1985; Leippe and Renwrantz, 1988).

Lysozyme has been demonstrated in hemocytes and hemolymph of bivalve molluscs (Cheng and Rodrick, 1974; Rodrick and Cheng, 1974; Cheng, 1981). It was identified in the hemolymph of oysters which lysed certain gram-positive bacteria (*Bacillus megaterium* and *Micrococcus lysodeikticus* (McDade and Tripp, 1967a,b). Other serum enzymes (e.g., β -glucuronidase, acid phosphatase, alkaline phosphatase, lipase, and nonspecific esterase) may act on compatible substrates, including pathogens (Cheng and Rodrick, 1975; Cheng and Yoshino, 1975). The pattern of enzyme activity during phagocytosis involves stimulation by certain antigens, resulting in enhanced lysosomal enzyme synthesis, with their subsequent release in the hemolymph by degranulation (Cheng, 1981, 1983a,b; Cheng *et al.*, 1975). From studies to date, it seems that destruction of susceptible bacteria by elevated levels of lysosomal enzymes in hemocytes and hemolymph is a principal defense mechanism in bivalve

molluscs (Cheng, 1981). Cheng's summary of bivalve humoral defenses includes the following points:

1. Evidence exists for acquired humoral protective molecules in bivalves.
2. These molecules are lysosomal enzymes of low specificity released from granulocytes into the hemolymph.
3. These molecules may be responsible, at least in part, for the antibacterial activity of bivalve hemolymph.

Cheng pointed out, however, that low levels of lysosomal enzymes do occur in unchallenged bivalves, and that much bacteriolytic activity occurs within the hemocyte as well.

An important criterion of the degree of effectiveness of internal defense mechanisms in molluscs has been described as "immunological memory." Defined as "the alteration of the immune response resulting from previous exposure to an antigen" (Bayne *et al.*, 1980), the evidence in bivalve molluscs is not too impressive, but some information does exist. Primary responses—heightened titers of antibacterial agglutinins in the hemolymph after initial exposure—have been demonstrated in oysters (Hardy *et al.*, 1977), but not in several gastropods tested. Secondary responses, measured by faster clearance of injected bacteriophage, were reported in oysters following repeated challenge (Acton and Evans, 1968; Acton, 1970), but were not seen in gastropods tested (Nelstrup *et al.*, 1968). That the more rapid clearance was not specific was suggested by the fact that primary stimulation by an antigenically unrelated phage still produced accelerated clearance when oysters were later challenged with the T₂ coliphage.

Evidence for inducible humoral protective factors in molluscs must therefore be described as inconclusive. For example, an inducible bactericidin could not be demonstrated in oysters (Weinheimer *et al.*, 1969a). On the other hand, significant elevation in the intracellular aminopeptidase activity of oyster hemocytes was induced by *in vitro* exposure to heat-killed *B. megaterium* (Yoshino and Cheng, 1976).

In another study three species of abalones injected with gram-negative bacilli exhibited increased hemolymph bactericidal activity above that of noninjected controls (Cushing *et al.*, 1971). The response was transient, however, and disappeared in 5–8 days.

An important point about molluscan humoral responses to infection was made by Feng and Canzonier (1970): that failure to demonstrate such responses is often because experimental animals are injected with a variety of fluids or particles, but examinations of humoral responses to *natural infections* have not been pursued as vigorously—especially the

use of model host-parasite systems, which in some instances *do* indicate responsiveness.

C. Resistance of American Oysters to Infections by *Haplosporidium nelsoni*: A Case History

The extensive and continuing mortalities of oysters in Delaware and Chesapeake bays caused by a haplosporidan disease have encouraged field and experimental studies of resistance to the pathogen. Significant new information about responses to infections has accumulated, especially from a series of studies by scientists at Rutgers University.

Probably the most important finding (Ford, 1985a) was that, under conditions of high mortality due to a virulent pathogen (*Haplosporidium nelsoni*), with consequent high selection pressure for increased disease resistance, *the course of infection changed from acute, with high mortality (up to 95%), to chronic, with fewer (approximately 50%) mortalities*. The precise mechanism is still unknown, but some resistant animals seemed able to contain and localize infections, preventing them from becoming systemic (Ford and Haskin, 1982). In other resistant animals, however, chronic infections did become systemic, but the animal seemed able to tolerate heavy parasite burdens (Ford, 1985a). One observation of undoubted significance is that the host cellular response (as measured by hemocyte infiltration and phagocytosis) was quantitatively the same in selected and unselected individuals. Resistance in oysters, therefore, is *to mortality induced by the disease*, rather than *to infection by the pathogen, Haplosporidium nelsoni*. Stated another way, "Resistance to MSX [*Haplosporidium nelsoni*]-kill in native oysters is not correlated with an ability to prevent infection, but with restriction of parasites to localized non-lethal lesions. Many survivors are able to suppress or rid themselves of infections when temperatures approach 20°C in late spring" (Ford and Haskin, 1982, emphasis added). Furthermore, infected resistant oysters seemed able to tolerate parasite burdens that would kill susceptible oysters (Ford and Haskin, 1987).

The resistance of survivors of *Haplosporidium nelsoni* mortalities in Chesapeake Bay (Marumsco Bar) had been proposed earlier by Farley (1968, 1975). He examined a disease stage labeled "remission," characterized by abatement of cell infiltration, large numbers of pigment cells in connective tissue, inactive moribund-appearing parasites, decrease in parasite numbers, phagocytosis of parasites, localization of parasites in and near epithelia, and a progressively lower prevalence and intensity of infection in populations, without noticeable mortalities. These remissions were considered to be indications of resistance or immunity; they

occurred with greater frequency in survivors of 5 years of exposure to the disease than in newly introduced susceptible stocks. Survivors with remission characteristics often had new shell growth and were in good condition, and parasites were either absent or confined in localized lesions near the mantle. Some oysters with signs of earlier infection and remission were also seen with localized, inactive gill lesions, suggesting to Farley reinfection but containment of the new infections. He also observed massive increases in parasite intensity following remission, characterized by heavily infiltrated lesions containing proliferating parasites. This type of infection was thought to represent "relapse" of the disease, due to the return of favorable conditions for the parasite in oysters which had previously undergone remission (Farley, 1968).

One major advantage of the Rutgers studies in Delaware Bay, mentioned earlier, was that they included the examination of resistance to *Haplosporidium nelsoni*-caused mortality in both laboratory-reared and native populations of oysters (Haskin and Ford, 1979). Investigations began in 1958, the year after disease-caused mortalities were first observed in Delaware Bay, and have continued to the present time. Early evidence from studies of native populations that resistance was developing among survivors was summarized by Haskin and Ford as follows:

- From 1958 to 1960 successive year classes of spat subjected to heavy disease pressure showed increasing survival with each year class (from 16% in the 1957 set to 71% in the 1959 set).
- Beginning in 1961 planted seed oysters showed improved survival.
- Native Delaware Bay stocks had higher survival rates than did oysters imported from disease-free East Coast locations.
- Histopathological studies disclosed that higher survival rates were correlated with lighter, more localized infections, suggesting a defense mechanism resulting in containment of the parasites.
- Cumulative mortalities for all planted oysters sampled between 1960 and 1977 averaged 36% after 1 year, 50% after 2 years, and 56% after 3 years.
- No further decline in mortalities has been noted since the mid-1960s; mortalities fluctuate with activity of the pathogen, as influenced by temperature and salinity; losses have not equaled those of the late 1950s, when total 2-year cumulative mortalities reached 90–95% over most of the planted beds.

Beginning in 1961 laboratory rearing of oyster stocks with varying histories of exposure to *Haplosporidium nelsoni* infection was instituted

(Haskin and Ford, 1979). Evidence acquired during the subsequent two decades indicated that "resistance is heritable, and can be expressed to a high degree in laboratory-reared oysters with rigorously selected parents." Among the most significant findings of the long-term selection experiments are these (as summarized by Haskin and Ford, 1979):

- "Examining cumulative mortalities of three generations of selected stocks, a clear pattern of differential mortality can be seen (Fig. 39). Resistant laboratory-reared oysters exposed to the disease for 3 years had mortalities of 56%, compared with 81% in Delaware Bay native oysters and 93% in susceptible stocks from disease-free areas on the east coast of United States."
- "Calculation of survival ratios disclosed that after 3 years of intensive selection, third generation resistant laboratory stocks had 6.3 times as many survivors as susceptible stocks, while native Delaware Bay stocks had nearly three times as many survivors as susceptible stocks."
- "Susceptibles reared from stocks imported annually from disease-free areas had a total 2-year cumulative mortality level of 92%—comparable to that of Delaware Bay natives during the first 2 years of the epizootic which began in 1957."
- "Even after three generations of severe selection pressure by exposure to the Delaware Bay environment, *Haplosporidium nelsoni* still killed oysters of the F₃ generation, suggesting that resistance is continuing to develop in laboratory-reared stocks."

A straightforward genetic explanation was proposed by Haskin and Ford (1979) for the development of resistance to *Haplosporidium nelsoni* by *Crassostrea virginica*. Their principal points were:

- "A population of oysters which has never been exposed to MSX [*Haplosporidium nelsoni*] contains a random distribution of those genes which will determine its capacity to deal with the disease. These genes present a continuum of abilities ranging from total incapability in highly susceptible individuals, through stages of increasing ability to control infections, to some highly resistant oysters well-equipped to deal with intense MSX pressure."
- "Upon first exposure to MSX, the highly susceptible individuals, which comprise most of the population, die. Some survivors of the initial kill also contain susceptible characteristics, but these are masked to varying degrees by resistant ones. Such individuals are infected with MSX, however, and are weakened by lingering infections, renewed infections, and non-MSX stresses."

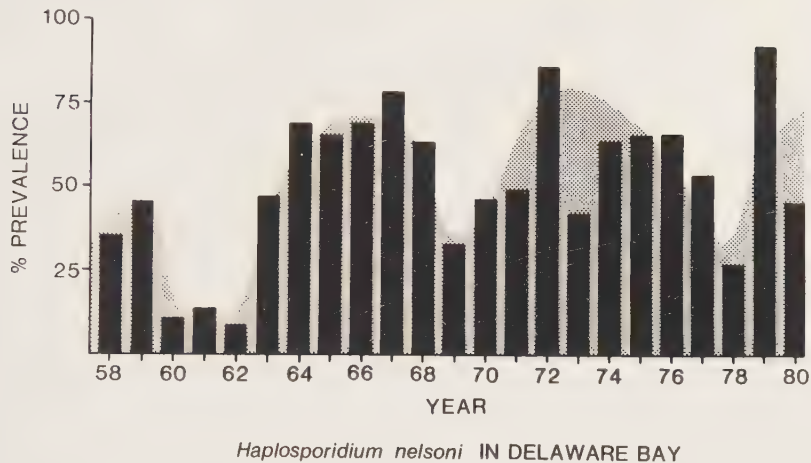


Figure 39. Cumulative mortality means for selected oyster stocks exposed to *Haplosporidium nelsoni* in Delaware Bay. Modified from Haskin and Ford (1979).

There is, following the initial massive kill, a progressive ‘weed-ing out’ in which oysters with the lowest proportion of suscepti-ble genes survive longest. At the end of the 33-month test peri-od, survivors with a preponderance of resistant genes still have some masked susceptible qualities.”

- “In reproduction, genes are recombined and some offspring ac-quire a greater proportion of susceptible characters than their selected parents had. Upon first exposure to MSX, these die and there follows the same gradual selection of less susceptible indi-viduals as in the parent generation.”
- “The argument for such a recombination is strengthened by sea-sonal mortality patterns [Fig. 40]. There is a consistently higher mortality during first exposure in any given generation than there was in the parents during their last season of exposure.”
- “With each succeeding generation, fewer susceptible traits re-main to be passed along, and MSX-caused mortalities decrease accordingly.”

In two field experiments transferring infected Delaware Bay juve-niles to a disease-free site on the northern New Jersey coast (Monmouth Beach), significant new information was obtained about the course of *Haplosporidium nelsoni* infections in the absence of reinfection (Ford, 1985a):

- Oysters, once infected, may remain infected for several years.

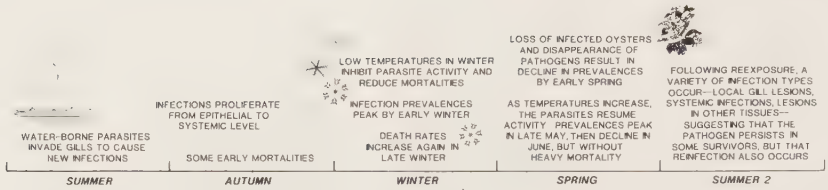


Figure 40. Seasonal sequence of *Haplosporidium nelsoni* activity in Delaware Bay. Modified from Ford (1985a).

- A substantial proportion of infections become chronic.
- Seasonal patterns of the disease indicate a major influence of temperature. The seasonal pattern of infection in individual oysters, in the absence of reinfection, followed a temperature-related sequence of localization in gill or gut epithelium, relapse into generalized infections, then localization again.
- Some infections that were “lost” in May–June were apparently temporary remissions that later relapsed into patent infections.
- The role of phagocytes seems to be one of removing degenerating pathogens rather than defense against live pathogens.
- The typical spring peak of prevalences may be the consequence of relapsed and newly acquired infections.
- Chronic infections debilitate oysters and probably reduce capacity to tolerate other environmental stressors.

The demonstration that long-term chronic infections may occur places a whole new perspective on the progression of the disease, since formerly infections were thought to progress at varying rates and kill the host.

Another series of experiments conducted earlier by the Rutgers group (Myhre and Haskin, 1968, 1970; Myhre, 1973) demonstrated the effects of elevated temperature on the resistance of oysters to *Haplosporidium nelsoni* infections. Comparing susceptible and resistant stocks, the authors found that, in susceptible stocks, infections intensified rapidly with increasing temperature, and mortalities ensued. In selected (resistant) stocks, infections decreased in intensity and number, with no concomitant mortality (Ford and Haskin, 1982). In a related study (Haskin and Douglass, 1971; Douglass, 1977) infected oysters were held at various temperatures from 5 to 22°C. Those held at 10 and 15°C showed an initial increase in prevalence, then no change, while those held at 18.5 and 22°C showed an early increase, followed by decline in prevalence and intensity—suggesting to the authors that mechanisms of resistance were more effective at the higher temperatures. That hypothesis was supported by data from Ford and Haskin (1982), in which many

infections declined and disappeared as seasonal temperatures exceeded 20°C.

Haplosporidium nelsoni infections can provide an example of one form of disease resistance in oyster populations. Chronic infections in resistant survivors are characterized by hemocytosis, poor condition, lowered resistance to stressors, and elevated death rates. Defenses may decline as a consequence of repeated challenges by new infective particles or by prolonged low-level chronic infections (Ford, 1985a).

A long-term program of breeding resistant oysters was also carried out in lower Chesapeake Bay (Andrews and Frierman, 1974; Andrews, 1984). After 15 years of intense selection, survivors exhibited strong resistance to *Haplosporidium nelsoni*; infections were few, and annual mortalities were fewer than 10%. Over 100 separate populations from various brood stocks were tested by field exposure for 2–3 years (Andrews, 1984).

Salinity has been shown to have a significant role in the course of infection with *Haplosporidium nelsoni*. A 20-year study reported by Haskin and Ford (1982) and by Ford (1985b) in Delaware Bay provided evidence that the prevalence of the pathogen decreased regularly from high to low salinity, but that infection *intensity* showed a sharp drop when salinities decreased below 12 ppt—suggesting a salinity threshold for the parasite that has little effect on the distribution of infective stages or their ability to infect, but that limits the activity and the lethality of the pathogen once infection has occurred. Acquisition of infections is rare below 9–11 ppt (Haskin and Ford, 1982).

The information from Delaware Bay is supported by data collected from Chesapeake Bay, where prevalences of 30% at one location (Wreck Shoal in the James River) persisted in autumn and winter at salinities of 7–15 ppt, but disappeared histologically in the spring, when salinities dropped to 2 ppt (Andrews, 1964). Similarly, Sprague *et al.* (1969) found in experimental studies that infected oysters placed in 7–8 ppt salinities for 6 months apparently lost the infections. Haskin and Ford (1982) reported a complete loss of infections in oysters transferred from high-salinity water to two locations where values ranged from 7 to 16 ppt and from 3 to 9 ppt. Infections gradually became lighter before they disappeared. Of course, the absence of the parasite in histological sections does not negate its possible persistence at a level below detection limits in tissues, or in a form not previously associated with the disease.

Salinity relationships of *Haplosporidium nelsoni* were summarized by Haskin and Ford (1982) as follows:

- 10 ppt: This is a minimum level for parasite survival.
- 10–20 ppt: Infections are acquired in a pattern paralleling the

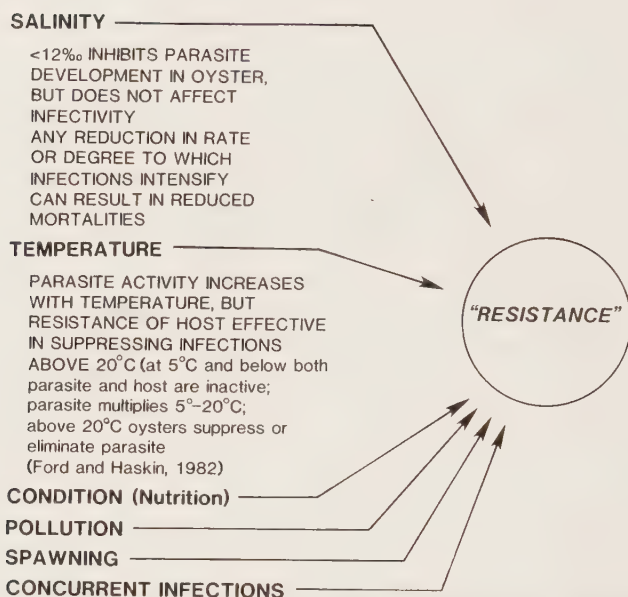


Figure 41. Environmental and physiological influences on the resistance of oysters to the protozoan pathogen *Haplosporidium nelsoni*.

salinity gradient, but the development of infections and consequent mortality are inhibited.

- 20–30 ppt: Full parasite activity is manifested.
- 30 ppt: Parasite activity may decrease, but findings seem equivocal.

The authors pointed out that these values were estimates, not exact thresholds.

At any rate, it is apparent from these data that resistance of oysters to the disease may be modified drastically by temperature or salinity, or by interactions of the two factors (Fig. 41).

Field and laboratory studies have helped to clarify some aspects of resistance to *Haplosporidium nelsoni* infections in oysters (Haskin and Ford, 1987), but the specific mechanisms of immunity in oysters have proved more elusive. Phagocytosis and encapsulation have been proposed as primary cellular responses of molluscs to the presence of foreign antigens (Cheng, 1981; Bayne, 1983), but both are apparently ineffective in *Haplosporidium nelsoni* infections of *C. virginica* (Ford, 1986). Phagocytosis seems to be principally to remove moribund or dead parasites, and encapsulation does not occur (although intense hemocytic

infiltration of tissues around the pathogen does occur). Confronted with the absence of clearly defined and effective cellular defense mechanisms to combat *Haplosporidium nelsoni* infections, attention has been focused on humoral constituents. A recent study of hemolymph proteins in susceptible and resistant laboratory-reared stocks disclosed that total serum protein concentrations decreased in proportion to disease intensity in oysters with systemic infections (Ford, 1986). Heavily infected individuals had only one third of the total protein of uninfected animals, and protein depletion was suggested as contributory to the death of heavily infected oysters. Serum protein levels in resistant individuals, with infections localized in the gill epithelium, were not depressed. Ford concluded that low values for hemolymph proteins were the consequences of long-term systemic infections and not due to interference with feeding by parasite-induced gill lesions. The results of the study are at variance with those reported earlier by Feng and Canzonier (1970), who found hemolymph total protein "not greatly altered in infected oysters," but consistent changes were seen in electrophoretic patterns of diseased and normal oysters, which the authors thought were indicative of a host humoral response to infection.

Alterations have also been noted in other hemolymph components of infected oysters. Lysozyme, free amino acids, and phosphohexoisomerase have been reported to be correlated with *Haplosporidium nelsoni* infection (Feng and Canzonier, 1970; Feng *et al.*, 1970; Mengebier and Wood, 1969; Cheng and Rodrick, 1975). Individual and temporal variability made interpretations difficult, but interference with normal metabolic processes of the oyster was indicated—particularly interference with active syntheses or competition for metabolic substrates. In a later study hemolymph enzyme activity was reported to increase sharply during the gill lesion stage of infection, but was not significantly elevated during the general infection stage (Douglass and Haskin, 1976; Douglass, 1977).

Agglutinins for mammalian erythrocytes have been found in oyster hemolymph, but antibacterial agglutinins were not seen (Tripp, 1966, 1974), nor have agglutinins reactive with *Haplosporidium nelsoni* been reported.

One clue to the nature of resistance in *C. virginica* to *Haplosporidium nelsoni* infections was alluded to by Douglass (1977) and by Ford (1986) and emphasized by C. A. Farley (personal communication, 1987)—that oyster hemocytes were phagocytizing *damaged or dead* parasites. This suggests that *Haplosporidium nelsoni* developmental stages were being killed in the host tissues, by factors as yet unknown. Lethal factors may be related to lytic properties, natural and inducible, that have been

demonstrated in oysters and other bivalve molluscs (McDade and Tripp, 1967a,b; Anderson, 1981).

The present knowledge about resistance of oysters to *Haplosporidium nelsoni* infections has been summarized admirably in a review by Ford (1988). Her principal points are:

- Survival mechanisms in oysters have been found (1) to be innate (appearing upon first exposure to the pathogen); (2) to restrict pathogens to small nonlethal lesions, usually of the gills and the palps; (3) to include elimination of some pathogens by phagocytosis and diapedesis; and (4) to be less effective at low temperatures (below 18°C). Furthermore, experimental results indicate improved survival of resistant oysters through *tolerance* to systemic infections during periods of low temperature, as well as through the *restriction* of parasites during periods of high temperature.
- The ability to restrict and tolerate infections is limited; eventually, most resistant oysters in an enzootic area die from stresses of chronic and repeated reinfections.
- The responses of oysters to infection are characterized by intense infiltration by hemocytes—especially hyalinocytes—into tissues invaded by the parasites, probably in response to tissue damage rather than to the presence of the pathogen. Hemolymph protein and enzyme levels may be altered, but humoral responses have not been associated with recovery from infection.
- Of possible significance to survival are physiological and metabolic conditions in the host, which provide or fail to provide a suitable environment for parasite development.

The excellent body of information about disease resistance acquired by the Rutgers group must be considered a classical long-term study in oyster pathology. Despite this extensive work, augmented by information from other research sources, questions still to be resolved include those concerning innate versus acquired resistance. Will increased resistance persist in the absence of high infection pressure and constant reinfection, or will it decline after a few generations of nonselection? Also, is there any element of *individual* response to infection, so some level of *acquired* resistance is achieved? There is general consensus that invertebrates as a group tend to be less specific in their responses to exposure to foreign antigens, but there is also some limited information indicating that bivalve molluscs *do* respond to infections—with higher levels of antibacterial activity in the hemolymph of previously exposed animals (Mori *et al.*, 1980a,b, 1984).

Although the story of resistance of oysters to infections with *Haplosporidium nelsoni* is undoubtedly the best documented among the diseases of *C. virginica*, some information is available about resistance to two other diseases: Malpeque Bay disease, caused by an unknown pathogen; and "Dermo" disease, caused by the apicomplexan protozoan, *Perkinsus marinus*.

Despite the serious and persistent handicap of inability to identify an etiological agent, studies of Malpeque Bay disease conducted sporadically since 1915 (Needler, 1931; Needler and Logie, 1947; Logie, 1956; Logie *et al.*, 1960) indicated the development of resistance after a period (15–20 years) of severe selection pressure from the unknown pathogen. Resistance developed gradually in the years following initial mortalities, which exceeded 90% in all oyster-producing areas of Prince Edward Island, beginning in Malpeque Bay. Resistance was signaled by slowly decreasing spat mortality and total mortality, and by continued high mortalities in introduced susceptible stocks.

An interesting aspect of Malpeque Bay disease resistance is that it enabled the revival of an oyster fishery on the north shore of New Brunswick, following mortalities there (apparently caused by the same pathogen) which began in 1950 and peaked in 1955. Mass transplants of resistant oysters to depleted New Brunswick beds from Prince Edward Island stocks took place in 1957, 1958, and 1959. After an acclimation period of 1 year, the transplants spawned, and spat survival was good. The fishery recovered, but never reached former levels of production.

Evidence for the resistance of oysters to the apicomplexan *P. marinus* is thin. Some preliminary experiments indicate that oysters resistant to *Haplosporidium nelsoni* infections are also resistant to *P. marinus* (Valiulis, 1973; Valiulis and Haskin, 1973, abstract). Laboratory-reared stocks of *C. virginica* with known resistance to *Haplosporidium nelsoni* were injected with graded doses of *P. marinus*-infected tissue. Of the four experimental groups, 89% of the oysters that died were infected with *P. marinus*. At low dosages of *P. marinus* (ten to 100 cells per oyster), two stocks that were most resistant to *Haplosporidium nelsoni* were also most resistant to *P. marinus*, whereas the stock least resistant to *Haplosporidium nelsoni* was also least resistant to *P. marinus* (as measured by cumulative mortalities). At higher dosages of *P. marinus* (500–10,000 cells per oyster), no differences in resistance could be detected. These findings should be extended.

This interesting insight into the possible resistance of oysters to two severe pathogens is obscured, however, by their epizootiology in Chesapeake Bay. *Haplosporidium nelsoni* replaced and displaced *P. marinus* as a major cause of oyster mortalities in lower Chesapeake Bay in the

1960s, despite the earlier presence and effects of *P. marinus*. This may have been because major mortalities due to *P. marinus* are thought to have occurred in the area sporadically since 1929, and the continuing enzootic presence of the pathogen may have led to the development of resistance to *P. marinus* infection.

Geographic differences exist in resistance to *P. marinus*. South Carolina oysters were reported as being more resistant than lower Chesapeake Bay oysters (Andrews and McHugh, 1956), while oysters from the outer peninsular coast of Virginia were less resistant than were Chesapeake Bay stocks.

Additionally, age differences may exist in susceptibility to *P. marinus*. Spat and oysters less than 2 years old have lower infection levels (Andrews and Hewatt, 1957). Spat less than 1 year are not usually killed, even though light infections may be acquired soon after setting (Mackin, 1962). Intensity of the disease increases with age until oysters are 3 years old (Andrews and Hewatt, 1957).

Studies of the resistance of oysters to *P. marinus* infections are also complicated by major effects of salinity on the course of infections. *Perkinsus marinus* is considerably more tolerant of low salinities than is *Haplosporidium nelsoni* (Haskin and Ford, 1982). Salinities as low as 11 ppt did not inhibit the development of the pathogen (Ray, 1954; Mackin, 1956), and infected oysters moved to salinities below 13 ppt did not show reduced disease activity or reduced mortalities when compared to high-salinity controls (Andrews and Hewatt, 1957). An outbreak of *P. marinus* has occurred in oysters in the low-salinity waters of upper Chesapeake Bay, leading some observers (Otto and Krantz, 1977) to speculate that a new strain of the pathogen, adapted to low salinities, may have been a factor in the resurgence of the disease. An alternative explanation may be that *P. marinus* is known to be much harder to eradicate by low salinity than is *Haplosporidium nelsoni*; therefore, it could exist in a low enzootic state until years of low rainfall and resultant high estuarine salinities.

Temperature is another environmental variable that may complicate attempts to elucidate the resistance of oysters to *P. marinus*. Infections appear to be lost during the winter, but there is some evidence that the pathogen persists in host tissues. Oysters from which infections had disappeared (i.e., samples were negative with thioglycolate culture) with the onset of cold weather were held in aquaria at 23–28°C; some of the oysters developed typical infections, which were transmitted to other individuals (Andrews and Hewatt, 1957). In another study oysters which had lost their infections during the winter and were thioglycolate negative were transferred to low-salinity waters where *P. marinus* did

not occur. Some developed infections during the following summer (Andrews, 1956).

Phagocytosis followed by diapedesis has been proposed as the most effective defense of oysters against *P. marinus* infections (Mackin, 1962). With this, parasite cells are phagocytized in the host tissues and transported across epithelia into the gut or to the exterior. Diapedesis seems to be less effective during the summer, however, when parasites are multiplying rapidly—even though it may be useful in lightly infected oysters.

In summary, then, intraspecific variations have been demonstrated in resistance to three diseases of American oysters: *P. marinus* disease (Andrews and Hewatt, 1957), Malpeque Bay disease (Logie *et al.*, 1960), and *Haplosporidium nelsoni* disease (Haskin and Ford, 1979). These differences suggest that resistance has a genetic base, but they do not explain the mechanisms that prevent or enhance infections (Feng and Canzonier, 1970; Ford and Haskin, 1987). The existence of host responses that keep infections in control is indicated by early findings with *H. nelsoni* disease: (1) High levels of infections may persist in resistant oysters with low mortality rates; and (2) localized infections are common in survivors of repeated exposures to intense pathogen pressure (Haskin *et al.*, 1965; Feng and Canzonier, 1970).

Attempts to relate these observations to mechanisms of resistance are complicated, however, by the significant effects of environmental factors such as temperature and salinity on the host and the parasite—effects which may mask or override an expected disease pattern.

III. MECHANISMS OF INTERNAL DEFENSE IN CRUSTACEANS

Studies of the internal defenses of Crustacea have a discontinuous history, which began in the late 1800s. Elaborate studies, early in the 20th century, of phagocytes and humoral factors in the hemolymph have been extended with renewed vigor during the past 35 years. As is true for other invertebrates studied, phagocytosis of foreign particles by fixed and mobile cells in the crustaceans is augmented by naturally occurring bactericidins, lysins, and agglutinins. A few instances of experimental enhancement of titers of such humoral factors by previous exposure to foreign protein have been reported. Specificity of natural and experimentally enhanced humoral factors is much lower than that of vertebrate immunoglobulins, but probably such factors act synergistically with cellular protective mechanisms, as they do in the vertebrates.

Phagocytosis by fixed and mobile cells in the gills, pericardial sinus, and sinuses at the bases of appendages seems to be a principal cellular defense perimeter in many crustaceans. Efficacy of phagocytes in destroying invading microorganisms varies, depending on the species of microorganism, as well as host physiology and environmental factors. Phagocytic activity is enhanced by hemolymph factors, which, in addition to immobilizing and agglutinating the invading organisms, also sensitize them to phagocytosis. Hemolymph factors, most of which seem to be of cellular origin, may also have bactericidal or lytic activity, leading to extracellular destruction of microbial invaders. A few recent studies indicate that these effects may be enhanced experimentally by the injection of killed or living microorganisms.

The number of known microbial diseases of crustaceans is greater than that known in most other invertebrate groups, with the possible exception of the Mollusca and Insecta. The number and depth of studies concerned with internal defense mechanisms of Crustacea are similarly greater than those of most other invertebrate groups—again with the possible exception of the molluscs and insects. One microbial pathogen of crustaceans that has received adequate attention is *Aerococcus viridans*, a gram-positive coccus which causes a fatal septicemia in lobsters and is capable of infecting other decapods. An elaborate series of studies in several laboratories has elucidated many details of the host-parasite relationship and has also provided extensive information about the internal defenses of a number of the larger Crustacea. *Aerococcus* constitutes a test microorganism of choice for future studies of disease processes in crustaceans.

To provide some structure for this section on crustacean defenses, the same sequence used earlier for molluscs will be followed and augmented, with discussion of cellular (phagocytic), bactericidal, lytic, agglutinating, precipitating, phage clearance, and antitoxic responses. It should be obvious that these systems are not mutually exclusive and may often interact or even share components to protect the individual animal from invasion by potential pathogens. Largely for ease of description, the systems will be considered consecutively, even though many may act in concert. After this, a detailed examination will be made of crustaceans that has received adequate attention is *Aerococcus viridans*, a gram-positive coccus which causes a fatal septicemia in lobsters and is

A. Cellular Mechanisms of Defense

The earliest study of phagocytosis in Crustacea concerned infections of *Daphnia* by the fungus *Monospora bicuspidata* (Metchnikoff, 1884). Fungal spores in the hemocoel were phagocytized and digested; the rapidity

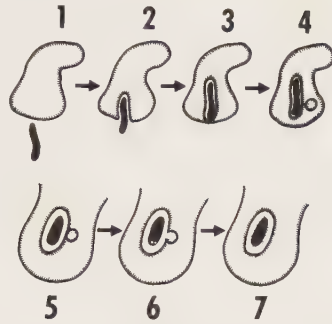


Figure 42. The role of the cell membrane and lysosome breakdown in phagocytosis. 1–4, phagocytosis; 5–7, lysosome activities. Redrawn from Hirsch (1965).

and vigor with which phagocytosis occurred determined the outcome of the infection. If some spores escaped phagocytosis, germinated, and formed conidia, the infection became generalized, and the host died in a few days. If all of the fungus spores were phagocytized and destroyed, the infection was arrested. Thus, the speed and the effectiveness of phagocytic action in some individuals, possibly mediated by humoral factors, determined survival. The absence of phagocytosis inevitably led to death.

Hemocytes of Crustacea were investigated long ago by Cattaneo (1888), Cuénot (1895, 1897, 1905), and Bruntz (1907). Cattaneo described the amoebocytes of *Carcinus maenas*; Cuénot reported blood-forming tissues—nodules of lymphoid-like cells in the blood sinuses—in decapods and described “phagocytic organs” in the hepatopancreas of decapods and amphipods; and Bruntz published an extensive paper on the hemocytes of many of the crustacean groups, distinguishing granular and hyaline hemocytes. Bruntz also described a “phagocytic organ” in gammarids. Other early studies of crustacean hemocytes include those by Hardy (1892), Tait (1918a,b), and Tait and Gunn (1918).

Following the classical early studies of Metchnikoff, Cuénot, Bruntz, and others, which elucidated the critical role of phagocytes in the internal defenses of crustaceans against microorganisms, phagocytic cells have received the greatest attention from vertebrate immunologists. General principles that have emerged from the more recent studies of phagocytosis in vertebrates undoubtedly apply as well to invertebrates.

The mechanism of intracellular degradation of phagocytized microorganisms has been described in general terms for the vertebrates (Fig. 42). Lysosomes—granules in the cytoplasm of phagocytes—contain antibacterial substances and hydrolytic enzymes (Cohn *et al.*, 1963). The

lysosomal membrane fuses with the vacuolar membrane within the phagocyte, releasing antimicrobial components into the vacuole (Robineaux and Frederic, 1955; Hirsch, 1965; Aarum, 1967). Invading microorganisms are subjected to antimicrobial factors both inside and outside the phagocytes. Substances of presumed cellular origin, such as lysozyme, occur in the phagocytes and the hemolymph.

In the vertebrates, specific and nonspecific serum proteins increase the speed and the effectiveness of phagocytosis—the opsonizing effect (Wright and Douglas, 1903; Suter, 1956; Rowley, 1960). Sensitization of bacteria with serum factors is not always a necessary prelude to phagocytosis, however, as was pointed out by Wood *et al.* (1946) and by Wood (1953). In the absence of other host responses, early phagocytic activity may be important in preventing infection.

Increases in phagocytic activity—a significant aspect of enhanced resistance to foreign antigens—has been reported in several of the larger Crustacea. The Australian crayfish, *Cherax destructor*, responded to injections of lipopolysaccharide by demonstrating increased phagocytosis of mammalian erythrocytes (McKay and Jenkin, 1970a,b), and the American lobster, *Homarus americanus*, injected with endotoxin or killed bacteria, responded with increased rate and efficiency of phagocytosis of sheep erythrocytes and bacteria (Paterson *et al.*, 1976). A detectable response began 8 days after exposure and persisted for at least 80 days (Paterson and Stewart, 1979). Activation of lobster hemocytes was also demonstrated *in vitro* by enhanced phagocytosis following lipopolysaccharide injection (Goldenberg *et al.*, 1984). Activation of crustacean hemocytes can thus be considered an immune mechanism analogous to activation of mammalian macrophages, as was pointed out by Goldenberg *et al.*

Phagocytosis, then, constitutes the keystone to resistance in crustaceans. As Aarum (1967) mentioned, “. . . the organism’s ability to oppose infection precisely follows the phagocytes’ ability to function optimally. Resistance is lowered by a lowering of phagocytic activity.” Phagocytosis can occur at the site of a lesion, in the filtering tissues and organs of the circulatory system, and (to a lesser extent) in the body fluid itself. Groups of fixed phagocytic cells are present in many crustaceans, most commonly in the sinuses and lacunae of the gills and at the bases of the appendages.

Phagocytes agglutinate, aggregate, and cooperate in defense, forming nodules (the *nodules leucocytaires* of Cuénot, 1898), which are also known in annelids, molluscs, echinoderms, and other invertebrates. In a number of animals, the nodules are brown, due to the presence of large numbers of brown granules in the phagocytes, which may be

excretion products or decomposition products. In *Gammarus*, for example, the phagocytes composing the nodules secrete a clear, yellowish, chitinous substance around the parasites. This secretion gradually becomes dark brown. The nodules appear as conspicuous black spots in infected individuals (Pixell-Goodrich, 1928) and are found frequently in the gills and appendages.

Hemocytes of crustaceans and other invertebrates also act in other ways to protect the individual from overwhelming microbial invasion. Hemocytosis and hemocytic infiltration have been described in a number of invertebrate groups (see, for example, Solangi and Lightner, 1976). Manifestations in invertebrates involve the proliferation of hemocytes, changes in the permeability of blood vessels, the leakage of body fluids into tissues, adherence of the hemocytes to blood vessel walls, and migration of the hemocytes into tissues around areas of injury or parasitic invasion.

An elaborate system of cellular responses to injury in penaeid shrimps was described by Fontaine and Lightner (1975). Included were:

1. Phagocytosis of necrotic or foreign material by two types of hemocytes—one with eosinophilic cytoplasm and a second which is a large macrophagelike cell
2. Encapsulation with many layers of hemocytes, the inner ones becoming flattened
3. Ingestion of foreign material by fixed phagocytes lining hemolymph sinuses
4. Infiltration of injured areas by fibrocytes which form a dense network of collagenlike fibers, which remain as a permanent scar
5. Formation of melanin in later stages of wound repair, in which the enzymes and the substrate are of hemocyte origin (Unestam and Nylund, 1972)
6. Elimination of foreign and necrotic material by the aggregation of phagocytes in gill lamellae and loss of terminal portions of lamellae at ecdysis

In all, six cell types were identified by Fontaine and Lightner (1975): three forms of hemocytes, fibrocytes, phagocytic cells of loose connective tissue, and fixed phagocytes lining blood sinuses.

Cytotoxicity of crustacean hemocytes—the killer cell function described earlier in this chapter for molluscs—has also been explored (Tyson and Jenkin, 1974; Söderhäll *et al.*, 1985). Hemocytes seem capable *in vitro* of secreting cytotoxic molecules. It has been postulated (at least

in some invertebrates, as in the vertebrates) that an effector-target cell contact is required to cause death of the target cell (Leippe and Renwanz, 1988).

The involvement of hemocytes in coagulation or clot formation is a complex one, inasmuch as either cellular or extracellular clots may be formed. Intravascular cellular clots adhere to the walls of blood vessels and spaces, producing stasis, and once they are formed, they persist for some time. Extracellular clots, resulting from release of the constituents of hemocytes, can inhibit microbial motion and thus render microorganisms more vulnerable to phagocytosis. Since Fredericq (1879) first pointed out that in Crustacea coagulation of hemolymph involves cell agglutination as well as plasma coagulation, others have demonstrated similar characteristics in a number of invertebrate groups. The release of a component from hemocytes and the role of this component in initiating coagulation of plasma were reported by a number of authors, beginning with Halliburton (1885). Löwit (1889) observed the rapid disruption of hemocytes and the rapid clotting characteristic of most Crustacea and concluded that a causal relation existed. Hardy (1892), Tait and Gunn (1918), Tyler and Scheer (1945), and George and Nichols (1948) all provided data which supported the conclusion that a component from certain hemocytes acts with plasma components to form clots. More recently, intravascular clotting has been observed in blue crabs, *Callinectes sapidus*, infected with the protozoan *Paramoeba perniciosa* (Johnson, 1976), and high mortalities in rock crabs, *Cancer irroratus*, were thought to be due to extensive intravascular clotting induced by the injection of *Vibrio* endotoxin (Newman and Feng, 1982).

Bang (1967a, 1968) demonstrated that in the hermit crab, *Eupagurus longicarpus*, cellular clots formed in at least two stages following injury—first a clumping and stickiness of hemocytes without change in shape or loss of granulation, then retraction of the clot and the development of a network of fibrous cell projections containing microtubules. Similar microtubules have been seen in clotting cells of several other marine crabs and in lobsters (Cohen *et al.*, 1977, 1983).

The abundant and elaborate earlier literature on hemolymph coagulation in Crustacea was admirably summarized and evaluated by Florin (1960). As he pointed out, coagulation has been considered by some authors to occur in two distinct phases—cellular coagulation and then plasma gelation—while other workers view coagulation as a continuous process in which plasma gelation begins around hemocytes. It was Florin's conclusion that plasma coagulation was a one-step process in which fibrinogen of the plasma was acted upon by a coagulin released by the hemocytes. He also reviewed the role of cellular clots in wound

repair, emphasizing the importance of secretion of a chitinous film over the wound area by underlying coagulated phagocytes.

Recent investigations of clotting processes in crustaceans (reviewed by Bauchau and Mengeot, 1978; Söderhäll, 1982, and Durliat, 1985) have indicated that hemolymph coagulation involves initial clumping of the hemocytes, followed by secretion of a coagulin into the plasma, which participates in the formation of an extracellular clot (Madaras *et al.*, 1979, 1981). In Decapoda, clotting factors exist in the cell-free plasma and in hemocytes. Plasma gel formation results from activation, by endotoxins, of serine protease enzymes (analogous to thrombin or trypsin) which act on the coagulogen. In lobsters the intensity of gelation is directly correlated with the numbers of circulating granular hemocytes (Durliat and Vranckx, 1981, 1982a,b). Clottable factors have also been reported in hemocyte granules of the lobster (Mengeot *et al.*, 1976, 1977) and the sand crab, *Ovalipes bipustulatus* (Madaras *et al.*, 1979, 1981).

Encapsulation is also a common form of cellular internal protection in invertebrates. Invading organisms, often relatively large, are surrounded by phagocytes and fibrocytes. The onset of encapsulation may be rapid, and the cellular aggregates may be resolved only very slowly. Melanization has been found to be important in the encapsulation reaction of some arthropods (especially in insects); cellular encapsulation of a parasite is followed frequently by melanin deposition on the outer surface of the parasite (Söderhäll, 1982). Melanization has been considered to constitute a defense reaction in Crustacea as well, since quinones, which are intermediate products of melanin synthesis, may act as bactericidal or fungistatic agents (Durliat, 1985). The role of melanin formation in invertebrate host defenses has been reviewed by Söderhäll (1982, 1986). Phenoloxidase, important in melanin formation, is present as a proenzyme in hemocytes and may be activated by a number of compounds, including bacterial endotoxins. Additionally, phenoloxidase has been proposed to have a much broader role in invertebrate immunity—including opsonic activity and participation in an integrated recognition system in arthropods (Taylor, 1969; Söderhäll, 1982).

A biochemical basis for many of the earlier observations on cellular mechanisms in Crustacea has been examined in great detail by Söderhäll and associates (Söderhäll, 1982, 1983, 1986; Söderhäll and Ajaxon, 1982; Söderhäll and Smith, 1983, 1986; Söderhäll and Häll, 1984; Häll and Söderhäll, 1982; Hergenroth and Söderhäll, 1985; Johansson and Söderhäll, 1985). A prophenoloxidase activating system, with similarities to that of vertebrate complement, has been proposed as a "recognition system" in arthropods.

In summary, then, the hemocytes function in a number of ways

beyond phagocytosis, although the latter must be considered the dominant cellular defense mechanism:

1. Hemocytes are important in cellular infiltration of injured or diseased tissue.
2. They are important in clotting—either as participants in cellular clots or by the release of secretions or injury products which combine with plasma components to form extracellular clots.
3. The hemocytes are of primary importance to encapsulation.

The blood cells of vertebrate and invertebrate animals have been investigated widely since the late 19th century, and the hemocytes of Crustacea have received at least proportionate study. Cellular defenses of Crustacea and other invertebrates are varied, but center on the hemocyte and its activities. Also important are the humoral defenses, which will be considered in the following section. Before proceeding to a discussion of other than cellular defenses, however, it seems relevant to include an often overlooked perimeter of defense suggested by Miles (1962). Early suppression of microbial numbers may be due to microbicidal activity of the tissue cells themselves, either innate or induced, or to soluble antimicrobial substances in the intercellular fluid of the integument. As Miles pointed out, such preinflammatory cellular defenses in no way diminish the importance of phagocytes and humoral factors, but only provide an added perimeter of defense. Antimicrobial capacities of tissues as a whole, and of nonphagocytic cells in particular, may be a mainstay of nonspecific resistance, in both primary invasion and the determination of the subsequent course of infection. Tissue defenses of this nature in the invertebrates may be of great significance.

B. Humoral Mechanisms of Defense

The early literature on humoral mechanisms of internal defense in crustaceans demonstrated that natural agglutinins and lysins, with some specificity, occur in a number of species and that relatively nonspecific responses to foreign antigens can be induced in certain instances. More recent information has improved the quality and quantity of data available, but has not provided new concepts or modified the general conclusions reached earlier.

Natural bactericidins have been reported in a number of marine invertebrates, including crustaceans (Bang, 1967b). Increases in titers of bactericidal activity after inoculation with formalin-killed bacteria were noted in West Indian spiny lobsters, *Panulirus argus*, and American lobsters, *Homarus americanus* (Evans *et al.*, 1968; Acton *et al.*, 1969b). In

experiments with American lobsters held in seawater at 5°C, the peak of bactericidal response against gram-negative bacilli was reached within 48 hours after inoculation, and high titers persisted throughout the observation period (11 days). In spiny lobsters, maintained at significantly higher environmental temperatures (26–28°), the primary bactericidal response to intracardial injection of living or killed suspensions of the same gram-negative bacilli (originally isolated from the digestive tract of spiny lobsters) reached a peak at about 36–48 hours after injection, then declined slowly for the following 2 weeks. A partial lack of specificity of the bactericidin was indicated by its appearance following the injection of gram-positive bacilli and by its activity against *Salmonella typhosa* and *Escherichia coli*, as well as against the unidentified gram-negative bacillus used as the homologous test organism. The bactericidin was not active, however, against *Pseudomonas aeruginosa* or three species of gram-positive bacteria.

A subsequent study of the specificity of the spiny lobster bactericidin further indicated that the response was partially nonspecific (Weinheimer *et al.*, 1969b–d). Low titers of the bactericidin against gram-negative bacilli could be demonstrated after injections of formalin-killed type 2 pneumococci and bovine serum albumin.

Secondary responses—those following reinjection of the same antigen after a lapse of time—of spiny lobsters to killed suspensions of gram-negative bacilli were also examined by Evans *et al.* (1969a,b). Titers of bactericidin were slightly but significantly higher after reinoculation than after primary inoculation, and the rate of secondary response (the number of hours to reach peak titer) seemed somewhat accelerated when compared with the primary response. Unfortunately, the number of animals tested was small. It seems important that similar observations be greatly extended, since, as the authors pointed out, the results were reminiscent of the specific anamnesis or immunological memory demonstrable in the immunoglobulin responses of vertebrates.

Noteworthy is that all of the American lobsters and a number of the West Indian spiny lobsters used in the studies by Evans *et al.* (1969a,b) had demonstrable preexisting titers of bactericidins against the gram-negative bacillus used in the experiments. The remainder of the spiny lobsters used in the studies could have had titers of bactericidins lower than were demonstrable by the methods used. Thus inoculation may not have “induced” the bactericidin, but instead may have merely enhanced or increased the titers. A number of explanations were offered for the preexisting titers of bactericidal activity, including trauma, because of handling, and response to previous bacterial infection. Two relevant observations are that in many American lobsters a rapid in-

crease in bactericidal titer preceded death, and in studies of spiny lobsters the bactericidal activity was found to be partially nonspecific, in that activity against other gram-negative bacteria was enhanced.

The conclusions reached by the research group that examined the spiny lobster bactericidin (Weinheimer *et al.*, 1969b) are in accord with findings for other phyla: "These data suggest that, although invertebrates appear capable of antigenic recognition, the molecules synthesized may have broad specificity covering a wide range of antigenic determinants. Further studies will be necessary to ascertain whether these inducible substances represent primitive immunoglobulins. In any event, it would be surprising if they did not have a major role in defense of the animal against pathogenic microbes."

An important qualification was pointed out by Aarum (1967) regarding results obtained by experimental inoculation of large numbers of bacteria. Infectious agents are found in large numbers in the circulatory system only in septicemia, which occurs only after the growth phase of a virulent pathogen within the host, often within fixed or immobilized phagocytic cells. A rapid increase in pathogen numbers in the circulating body fluid is usually a precursor to death and indicates a failure of host defenses. Thus, the artificial introduction of large numbers of microorganisms should not be expected to elicit normal responses in an animal.

Lytic activity is another obvious defense mechanism in crustaceans. A natural hemolysin for sheep erythrocytes in the hemolymph of West Indian spiny lobsters was reported by Weinheimer *et al.* (1969b). Low temperatures (0 and 4°C) inhibited lysis, which was best demonstrated at 25 and 37°C. Heating the lobster hemolymph to 52°C destroyed lytic activity. The hemolysin could be adsorbed on red blood cells or cell stroma at low temperatures. Erythrocytes with the adsorbed lysin were lysed when the temperature was raised to 37°C, which indicated a possible enzymatic type of activity of this hemolytic system. The authors suggested a multiple-step system, analogous to mammalian hemolytic systems, consisting of a single-protein species which is first adsorbed on the surface of the sheep erythrocyte and then—in one or more steps—lyses the cell.

A detailed study of the presence or absence of natural immunity to the invasion of the hemolymph of crabs by a parasitic ciliate, *Anophrys* (*Paranophrys*) *maggii*, was reported by Poisson (1930). The ciliate, which in shore crabs, *Carcinus maenas*, multiplies and causes death, was immobilized, agglutinated, and lysed *in vitro* by the sera of several other crabs—particularly *Maia squinado*, *Eupagurus prideauxii*, and *Portunus puber*. Experimental inoculations of ciliates were cleared usually within

several hours. It is interesting that the parasite, when experimentally introduced in another member of the Portunidae, *Portunus depurator*, multiplied and killed the experimental hosts just as it did in *C. maenas*. Poisson attributed the reactions of crabs to the ciliate to expressions of a natural immunity, effected by agglutinating and lytic activity of the hemolymph.

Other lytic systems of Crustacea have received passing attention. Cantacuzène (1913) found that hemolymph of the hermit crab, *E. prideauxii*, possessed a heat-labile hemolysin, as well as precipitating and agglutinating activities. Cantacuzène (1921, 1923) stated that injection of the spider crab, *M. squinado*, with sheep erythrocytes produced hemolysins as well as agglutinins.

Natural hemagglutinins of the Australian freshwater crayfish, *Cherax destructors*, were examined by McKay and Jenkin (1969, 1970a,b) and by McKay *et al.* (1969). Absorptions of hemolymph by erythrocytes of four mammalian and one avian species disclosed specificity, in that absorption by cells of one species still left agglutinins for cells of other species. *In vitro* studies with crayfish phagocytes and mouse erythrocytes disclosed that the crayfish hemagglutinins greatly enhanced the adhesion of the erythrocytes to the phagocytes—a specific opsonic effect (Tyson and Jenkin, 1973). These studies support earlier reports of hemagglutinins in a number of invertebrate phyla (Tyler and Metz, 1945; Tyler, 1946; Sindermann and Mairs, 1959; Cushing *et al.*, 1963; Tripp, 1966), with specificities somewhat comparable to those of vertebrate natural isohemagglutinins, and with some biological properties (such as enhancement of adhesion and phagocytosis of erythrocytes by phagocytes) similar to those of vertebrate antibodies. Recent studies suggest that the opsonizing and agglutinating activities of crustacean hemolymph serum may be discrete or at least separable quantitatively (Goldenberg and Greenberg, 1983). These authors reported a nonagglutinating carbohydrate-specific opsonin in the hemolymph of the American lobster, *Homarus americanus*.

A study of natural agglutinins in the serum of California spiny lobsters, *Panulirus interruptus*, for the blood and sperm cells of 54 species (representing seven phyla) was published in 1945 by Tyler and Metz, and is still noted as one of the most comprehensive examinations of its kind for a marine animal. Agglutination was not found in 37 other species tested. Titers for positive reactions ranged from 8 to 256. The most interesting phase of the study was an extensive series of absorptions of spiny lobster serum by cells of many of the species tested. In each case the absorption of serum with cells of any single species removed agglutinins for all of the species tested that belonged to the same

group (class), but left agglutinins for the cells of all other groups tested. Tyler and Metz concluded, on the basis of absorptions, that at least ten class-specific agglutinins were present in the serum of the spiny lobster. A few cross-reactions occurred, and some reduction in titers resulted from absorptions with cells of other species, which suggested the presence of a number of reacting sites on the cells.

Bang (1967b) examined the responses of the spider crab, *Maia squinado*, to injections of *Paranophrys maggii*, the large ciliate pathogenic for shore crabs, *Carcinus maenas*, as part of a laudable attempt to repeat with modern methods some of the early studies of invertebrate defenses. Hemolymph of some spider crabs strongly agglutinated the ciliates, but that of others did not. Agglutination resulted from formation of a mucoid substance around the tail cilia. Crabs that lacked the agglutinin died from overwhelming infections of the hemolymph, whereas those with the agglutinin survived (Bang, 1962). When present, the agglutinin was apparently fairly constant, except that in some crabs it was lost spontaneously but temporarily at the time of molting. Poisson (1930) had noted earlier that the hemolymph of *Maia* lysed the ciliates and that the hemolymph of the hermit crab, *Eupagurus prideauxii*, agglutinated them.

The serum of the American lobster contains strong, specific natural agglutinins for an antigen present on the red blood cells of sea herring, *Clupea harengus* (Sindermann and Mairs, 1959). Detection of blood groups in this fish was aided by the use of the hemagglutinin: individual herring either had the antigen or lacked it. Titers reached as high as 256, and reactions paralleled those obtained with absorbed rabbit antisera and plant lectins (Sindermann, 1963). Erythrocytes of other clupeoid fishes tested were also strongly agglutinated by lobster sera (Sindermann, 1962).

Production of *precipitins* by invertebrates has been reported only rarely. Osawa and Yabuuchi (1963), in a very brief paper, found that the "Homard americain, *Cambarus clarkii*" (probably *H. americanus*) did not produce agglutinins or lysins when injected with red blood cells but did produce weak precipitins (detectible by immunoelectrophoresis) after injection with sera from rabbits and goats. No information was given about dosage, injection schedules, time for response, or titers.

Stewart and Foley (1969) suggested that a "precipitin-like principle already present in the hemolymph" of the American lobster might be important in the removal of foreign protein. Fluorescein-labeled bovine serum albumin and lobster serum proteins were injected into lobsters held at 5°C, and the fluorescence level was checked periodically for 6 days. With lobster serum proteins an initial decline in fluorescence within 2 hours after injection was followed by a plateau that remained con-

stant for the duration of the experiment; with labeled bovine serum albumin, however, the rate of clearance was concentration dependent, but the foreign protein declined to very low levels by 3 days after injection. Fluorescent material was apparently excreted in proportion to its disappearance from lobster hemolymph. Pinocytosis by phagocytes was not demonstrated, but tiny granular fluorescent accretions were observed in the hemolymph of lobsters injected with labeled bovine serum albumin, beginning about 8 hours after injection. *In vitro* studies, in which a standard ring test with labeled or unlabeled bovine serum albumin and lobster serum was used, disclosed a clearly discernible ring after 16 hours at room temperature and a precipitate at the bottom of the tube after 36 hours. Precipitin titers of individual lobster sera ranged from 2 to 16 and were independent of the total protein concentration of the lobster serum. Dialysis of the lobster serum did not change the titers, but precipitin activity was destroyed by heating above 50°C.

A number of interesting implications of the study were pointed out by Stewart and Foley (1969). The clearance mechanism seemed able to distinguish between foreign and native proteins, and the capacity for clearance seemed high, as indicated by accelerated clearance of larger doses of bovine serum albumin. Attempts to increase the levels of precipitin by previous injection of lobsters with bovine serum albumin did not succeed and, in fact, resulted in decreased precipitin levels in some individuals. The authors suggested that the hemolymph factor responsible for the clearance of foreign protein may be maintained normally at low levels and may be supplemented by further secretions when required. Stewart and Foley also suggested that the precipitin principle in the hemolymph may be the first of several steps, or possibly the primary removal factor, and that digestion and excretion may take place elsewhere—probably in the hepatopancreas.

The results of the studies by Stewart and Foley (1969) are in agreement with those by Teague and Friou (1964), who observed that injected foreign protein was rapidly removed from the hemolymph of the crayfish *Cambarus virilis*. Previous injection of the protein did not increase the clearance rate. Teague and Friou did not observe precipitin activity against injected bovine and human serum albumins but concluded that clearance resulted from nonspecific degradation of the foreign protein.

Studies of *phage clearance* in crustaceans have produced confusing results. Taylor *et al.* (1964) presented some evidence (from two crabs) of an increase in the rate of secondary clearance of injected T₁ bacteriophage in the shore crab, *Carcinus maenas*. Clearance was not complete until after 2 weeks at 16–18°C, and no neutralizing antibody to T₁ phage was detected in the hemolymph. Primary inoculation with T₆ phage did

not increase clearance rates for T_1 secondary injections. The small number of animals used in these experiments makes the conclusions highly tentative. The authors suggested the existence of a "phylogenetically more primitive type of immune response than the production of humoral antibody," but did not state clearly what the response was—except possibly that it was "an apparently purely cellular secondary response."

Rapid T_2 bacteriophage clearance and the presence of a naturally occurring humoral factor which neutralized⁹ the phage were reported from hemolymph serum of blue crabs (*Callinectes sapidus*) (McCumber and Clem, 1977; McCumber *et al.*, 1979). Principal clearance occurred in the hepatopancreas, and some degree of specificity was indicated by the absence of clearance and neutralizing activity against T_3 and T_7 phages.

Other studies of phage clearance by Cushing and McNeely (as reported by Cushing, 1967) led to negative conclusions. Phage T_4 persisted for up to 168 days in the California spiny lobster and disappeared at a steady rate, uninfluenced by the size of the original inoculum. Two species of crabs tested also failed to clear bacteriophage. Other negative findings for an increased rate of phage clearance following inoculation in crayfish were reported by Teague and Friou (1964).

Little definitive information is available about *antitoxic activity* in invertebrates (except for responses to gram-negative endotoxin). As Huff (1940) pointed out, "Experimental demonstration of antitoxic action in invertebrates has failed for the most part because of lack of susceptibility of invertebrate cells to known toxins." Probably the best example of antitoxic phenomena in Crustacea was described by Cantacuzène (1925) and by Cantacuzène and Damboviceanu (1934a,b). The hermit crab, *Eupagurus prideauxii*, exhibited resistance to nematocyst toxin of *Adamsia palliata*, a commensal coelenterate commonly found on the shell of the crab. When injected, the toxin had no effect on *E. prideauxii*, but it was lethal to many other Crustacea and to a number of other invertebrates tested, including the closely related hermit crab, *Eupagurus bernhardus*. Cantacuzène also found that the serum of *E. prideauxii* could neutralize the coelenterate toxin when the two—serum and toxin—were mixed and injected into crab species susceptible to the toxin. The development of this antitoxic principle can be seen as a logical and necessary concomitant of the very close relationship of the crab and the anemone, but the question of whether this is an example of innate or acquired resistance has not been resolved. Another example of coelente-

⁹Neutralization can be defined as "the ability of plasma, serum, or fractions thereof to cause a depletion in bacteriophage plaque forming units" (McCumber *et al.*, 1979).

rate toxin lethal to crabs was reported by Lane *et al.* (1961). Biologically active peptides in *Physalia* nematocyst toxins were tested, using fiddler crabs, *Uca pugilator*, as assay animals.

Invertebrate responses to gram-negative bacterial endotoxins were the subject of a review by Levin (1967). The most striking activity of such endotoxins is the production, after experimental inoculation, of intravascular clots and the ensuing death of various crustaceans and other invertebrates. Antitoxic immunity has not been demonstrated, but, as Levin stated, "Endotoxin appears capable of activating complementary defense mechanisms in invertebrates, including aggregation of amoebocytes, coagulation, bacterial immobilization, and phagocytosis. All these may be operative through one type of cell—the amoebocyte." Most of these phenomena were demonstrated in a recent study of *Vibrio* infections in rock crabs, *Cancer irroratus* (Newman and Feng, 1982). Mortalities in experimental infections seemed to result from hemocyte clumping and intravascular clotting, possibly as a response to endotoxin.

C. A Disease of Lobsters Caused by *Aerococcus viridans*: A Case History

The American lobster, *Homarus americanus*, has an effective internal defense system, consisting of active phagocytosis as well as agglutinating and bactericidal (or bacteriostatic) activity, against a number of injected bacteria. The protective system seems to fail completely only when challenged by *A. viridans* var. *homari*, which is thus far the only bacterial pathogen known to develop systemic infections in lobsters and kill them. Probably the most extensive series of reports concerned with responses of invertebrates to a particular pathogen is that dealing with the lobster (and other decapods) and the highly pathogenic gram-positive coccus *A. viridans*.

Gaffkemia—the disease caused by *A. viridans*—is enzootic in *H. americanus* and has been reported to cause epizootics in captive populations of that species and the European lobster, *Homarus gammarus* (Roskam, 1957; Goggins and Hurst, 1960; Gibson, 1961; Stewart and Rabin, 1970). Microorganisms with characteristics of *A. viridans* have been isolated from shrimp (*Penaeus aztecus* from the Gulf of Mexico) and from crabs (*Carcinus maenas* and *Libinia emarginata* from New England and *Cancer irroratus* from eastern Canada), but gaffkemia is known only in lobsters. Early descriptions of the disease and its etiological agent (Hitchner and Snieszko, 1947; Snieszko and Taylor, 1947) have been followed during the past several decades by studies in several laboratories, which used the lobster and the pathogen as a test system to elucidate responses to infection and other aspects of the host-parasite rela-

Table 13. A Proposed Hypothesis to Explain the Course of *Aerococcus viridans* Infections in Lobsters at 15°C^a

Day	Development of Gaffkemia in Lobsters
0	Bacteria gain access to tissues of lobsters as a result of injury, which destroys the integrity of the exoskeleton (or possibly the gut epithelium). Lobster hemocytes phagocytize <i>A. viridans</i> .
2	Phagocytized bacteria may multiply within hemocytes. Hemocytes containing engulfed bacteria lodge in capillary and lacunar areas (e.g., heart, hepatopancreas, gills) of the lobster.
4	Hemocytes may be disrupted, releasing <i>A. viridans</i> in hemolymph. This may result in a rapid decrease in hemocyte numbers and a logarithmic increase in bacterial numbers.
6	Hemolymph stimulates multiplication of released bacteria. Hemocyte numbers seem to be gradually reduced by continued phagocytosis and disruption of phagocytes.
8-10	The clotting mechanism (release of coagulin from hemocytes) is affected—possibly by a reduction of hemocyte numbers—and clotting time is greatly prolonged.
12-14	Lobsters die from depletion of nutrient stores and utilization of this material by <i>Aerococcus</i> . Injured gaffkaemic lobsters may bleed to death. (The possibility of exotoxin has not been entirely eliminated, but there is no present evidence to suggest its existence.)

^a From Stewart (1980).

tionship. The probable course of infection in lobsters is summarized in Table 13, and the sequence of responses to infection is listed in Table 14. Snieszko and Taylor (1947) first satisfied Koch's postulates for the pathogen and demonstrated high mortality following inoculation of cultured *A. viridans*.

Studies by Rabin (1965) and by Rabin and Hughes (1968) confirmed that lobsters inoculated with *Aerococcus* became septicemic within 2 days and died a few days later. Inoculation of gram-negative endotoxin 10 hours before exposure to the pathogen did not alter the course of infection. Prior inoculation of heat-killed *Aerococcus* cultures (24 hours before challenge) produced no protection.

In vitro studies with lobster serum as a culture medium disclosed that growth of the pathogen was stimulated, while growth of a *Vibrio* (nonpathogenic to lobsters) was usually inhibited. Serum from lobsters which had been inoculated 24 hours earlier with killed *Aerococcus* still stimulated growth of the pathogen *in vitro*.

Rabin and Hughes (1968) tested the resistance to *Aerococcus* in a variety of studies with lobsters and other marine arthropods. Findings

Table 14. Sequence of Events in Lobster Response to Gaffkemia^a

-
1. Entry of virulent pathogen (*Aerococcus viridans*) through a break in integumental barrier.
 2. Pathogen enters hemolymph containing:
 - Inducible bactericidin
 - Agglutinins
 - Phagocytic hemocytes
 - Clotting mechanism
 but
 3. Bactericidin, activated by elements from hemocytes, is effective against many bacteria, but not *A. viridans*.
 4. Agglutinins vary seasonally and agglutinate a wide range of bacteria, but not virulent strains of *A. viridans*, since they develop thick capsules.
 5. Phagocytosis, assisted by opsonic factors in hemolymph (possibly associated with agglutinins), occurs but does not kill virulent *A. viridans*.
 6. Clotting seals wound rapidly but is not effective in confining the pathogen.
 7. Inducible parts of lobster defenses are the phagocytic index of hemocytes and the hemolymph bactericidin level, induced by injection of *Pseudomonas perolens* or its endotoxin (Paterson and Stewart, 1979).
-

^a After Stewart (1984).

in studies with spider crabs (*L. emarginata*), rock crabs (*Cancer borealis*) and horseshoe crabs (*Limulus polyphemus*) were that most of the test animals cleared inoculated pathogens. *In vitro* studies with hemolymph disclosed either no apparent effect or only slight inhibition of growth of the pathogen by sera of spider and horseshoe crabs, and a slight stimulation of growth by sera of rock crabs.

The possible role of exotoxin was tested in lobsters by Rabin and Hughes with inoculation of filtrates of *A. viridans* cultures. The filtrate had no effect when it was injected into the abdomen, but injection into the major joint of the chela induced autotomy or abnormal movements in over 50% of the lobsters treated.

Evidence of resistance to gaffkemia was noted by Rabin and Hughes in a single lobster, which had been infected naturally before it was brought to the laboratory. Twelve days after capture the lobster was free of the pathogen. The animal was inoculated twice with increasingly larger dosages of the pathogen and cleared the bacteria within 6 days, but died on the 11th day following the second challenge. The reactions indicated a partial resistance and an ability in some individuals to recover from gaffkemia. It is interesting that serum from this presumably resistant lobster was similar to that of other lobsters tested in that it did not inhibit growth of *A. viridans in vitro*.

Studies carried on by Stewart and associates at the Halifax (Nova Scotia) Laboratory of the Fisheries Research Board of Canada (now the

Department of Fisheries and Oceans) have extended the work of Rabin and have provided the greatest number of contributions to the literature about the effects of the disease on lobsters (Stewart, 1975, 1980, 1984). In accord with earlier studies, infections usually were fatal, although rare individuals infected with *Aerococcus*-like organisms survived (Stewart *et al.*, 1966).

Cornick and Stewart (1968a) provided considerable relevant information about the host-parasite relationships of *Aerococcus* and lobsters. Experimental infections by inoculation, in which dosages as low as approximately five bacteria per lobster at 15°C were used, killed 90% of the test animals within 17 days. The absence of an effective host defense against the pathogen was strongly indicated by the fact that the mean time to death was almost constant, regardless of the dosage. Cornick and Stewart's studies disclosed additional facts that help to explain the pathogenicity of the bacterium to lobsters: It resisted digestion in phagocytes and multiplied in the hemolymph; its growth was stimulated *in vitro* by the serum of lobsters, while growth of several other bacteria was inhibited; and it was not agglutinated by lobster serum, although all other bacteria tested were agglutinated.

Presence in the lobster hemolymph of effective defenses against bacteria other than *A. viridans* was indicated by clearance within 30 days of inoculated suspensions of *Micrococcus conglomeratus*, *Micrococcus sedentarius*, *Achromobacter thalassius*, and *Gaffkya tetragenae*. Since several of those bacteria are closely related to *A. viridans* (which was not cleared), some specificity of the phagocytic or humoral protective mechanisms is strongly indicated. Natural agglutinins in lobster serum were demonstrated against all bacteria tested (six genera), except for all strains of *A. viridans*. Such agglutinins were of low titer, nondialyzable, inactivated at 56°C, and seemingly nonspecific (as suggested by the limited observation that a single absorption of serum by *Flavobacterium marinum* removed agglutinins for all other bacteria tested except *Brevibacterium* sp.). In a later study (Cornick and Stewart, 1973) higher agglutinin levels could not be induced by the injection of gram-negative vaccines.

Bactericidal activity is an important component of the internal defense system of lobsters, and several studies have examined its role (or absence of a role) in gaffkemia (Acton *et al.*, 1969b; Stewart and Zwicker, 1972; Mori and Stewart, 1978a,b). It can be induced by injection of the nonpathogenic *Pseudomonas perolens*; it is activated by a factor contained in hemocytes; much of its activity is localized in the hepatopancreas and the hemolymph; and it is effective against most bacteria, except *A. viridans*. In the study by Mori and Stewart (1978b), enhancement of bactericidal activity against *P. perolens* by injection was high, but the dura-

tion of the enhancement was only 2 weeks. Enhancement was not observed after administration of a vaccine prepared from *A. viridans*.

Cornick and Stewart's earlier (1968a) observations on phagocytosis of *A. viridans* are interesting and warrant further investigation. They found no phagocytized bacteria in hemolymph preparations 15 minutes after inoculation, but they did find fluorescent dye-labeled bacteria in hemocytes in heart, liver, and gill tissues of experimental lobsters soon after inoculation. Circulating hemocyte numbers were reduced significantly within 15 minutes after bacterial inoculation, but returned to normal levels after 5 hours. These data are in agreement with the statements by Maynard (1960), that phagocytes which have engulfed foreign material lodge in capillary and lacunar areas of the crustacean body, resulting in a reduction in the numbers of circulating hemocytes. Bang (1956) observed in tissues of *Limulus* injected with gram-negative bacteria a similar reduction in circulating hemocytes. In Cornick and Stewart's study, long-term infections of lobsters were characterized by the presence of black nodules containing *A. viridans* in tissue cells in the gills, swimmerettes, and ventral abdominal sinuses of the lobster. As Parry (1960) had pointed out earlier, this type of aggregation in gills is a common phenomenon in Crustacea. Poisson (1930) observed that, in the few crabs (e.g., *Carcinus maenas*) resistant to the parasitic ciliate *Paraphrys maggii*, masses of dead ciliates occurred in the branchial lacunae, pericardial sinus, and hepatopancreatic sinuses. Degenerating ciliates eventually formed brownish cysts. Cantacuzène (1923) observed a similar phenomenon in *Maia squinado* inoculated with bacteria. He pointed out that the lacunar tissue of the branchial lamellae of decapod crustaceans, with its many fixed phagocytes, acts as an extensive and effective bacterial filter.

Cornick and Stewart (1968a) suggested that the development of polysaccharide capsules by *A. viridans* in later stages of infection could be an important device that prevented destruction of the phagocytized bacteria and allowed multiplication of the pathogen. They pointed out, as evidence, that unencapsulated organisms grown in culture were actively phagocytized. As Stewart and Rabin (1970) later reported, however, the unencapsulated cultured bacteria were also virulent. It may be that the capsule forms soon after the organisms are injected into the host. This observation of survival and growth of phagocytized encapsulated bacteria in lobsters is a direct counterpart of the inability of vertebrate phagocytes to destroy many encapsulated microorganisms, and parallels the earlier findings by Cantacuzène (1923) of a fatal disease in crabs induced by encapsulated gram-positive bacteria.

Stewart *et al.* (1969a–c) examined certain insusceptibility factors af-

fecting *Aerococcus* disease in lobsters. Destruction of the integrity of the integument seemed essential for transmission of the pathogen. Acidity of the gastric fluid was bactericidal and appeared to provide an effective barrier against oral infection. Previous attempts to infect lobsters by feeding infected material had been unsuccessful (Snieszko and Taylor, 1947; Wood, 1965a,b; Rabin and Hughes, 1968).

Undoubtedly, such insusceptibility factors are of definite importance to the epizootiology of gaffkemia for several reasons: lobsters are cannibalistic, hemolymph of moribund gaffkemic individuals contains about 10^9 organisms per milliliter (Stewart *et al.*, 1969a-c), and the pathogen can be isolated consistently from lobster pounds, seawater, bottom mud, and slime from holding containers (Goggins and Hurst, 1960).

A thorough study of the effects of temperature on experimentally induced *Aerococcus* infections in American lobsters was reported by Stewart *et al.* (1969a-c). Mean time to death was inversely related to temperature. At 1°C no deaths attributable to experimental infections occurred, at 3°C mean time to death was 172 days, and at intermediate higher temperatures mean time to death decreased drastically to a minimum of 2 days at 20°C (which approaches the upper lethal temperature for American lobsters). It is important to note (in view of the very low seasonal temperatures of waters in which lobsters live naturally) that at 1°C the pathogens persisted in the host in low numbers but with virulence unchanged, and they produced mortalities when the temperature increased. Experimentally infected lobsters were also sensitive to and died from rapid increases or decreases in environmental temperatures, although the temperature changes used in the experiments were probably greater than those that would normally be experienced in nature.

A concurrent physiological and biochemical study by Stewart *et al.* (1969a,b), in which an attempt was made to define features of the infection that lead to death of lobsters, produced several interesting results. The pathogen lacked proteolytic, lipolytic, and fibrinolytic exoenzymes, suggesting that harmful effects are not caused by the direct destruction of tissue. The authors observed that, although *in vitro* growth of *A. viridans* was limited by the carbohydrate level of the lobster serum medium used, presumably such a level would be maintained *in vivo* at the expense of other tissues. Drastic reductions in hepatopancreatic glycogen and hemolymph nonprotein nitrogen characterized later stages of the infection. No evidence of a toxin was detected, and the conclusion was that gaffkemia is largely a wasting type of disease—that death from the disease was “a result of an unsuccessful competition on the part of the lobster for its own readily available storage material (Stewart *et al.*, 1969a).”

Vaccines prepared from avirulent strains of the pathogen have induced a degree of resistance (measured by increased time to death) to the virulent form, whereas vaccines prepared from formalin-killed virulent forms were less effective (Stewart and Zwicker, 1974a,b). Better results were obtained using heat-killed pathogens (Arie and Stewart, 1984). Best results were obtained by preliminary injection of the antibiotic vancomycin, followed in 24 hours by the injection of live pathogens. A high degree of resistance was produced (Stewart and Arie, 1974; Stewart and Zwicker, 1974a,b). The vancomycin may act as a metabolic inhibitor during the *in vivo* log growth phase of the pathogen, and cell wall synthesis seemed to be the target. Unfortunately, the resistance was transient. Challenge of resistant lobsters after 3 months produced 100% mortality, with a mean time to death identical to that for unvaccinated controls. In other studies, using avirulent strains of *A. viridans*, some degree of short-lived resistance to virulent strains was induced, as measured by an increased time to death and the survival of some challenged animals (Stewart and Zwicker, 1974a,b; Stewart, 1975). Repeated injections did not, however, produce higher degrees of resistance, and the precise mechanism of the induced resistance was not determined. (It might be noted here that a commercial vaccine against *A. viridans* has now been developed and licensed, according to an abstract published by Paterson *et al.* in 1988. Administration by injection and immersion routes was stated to result in increased recovery, in impounded lobster populations, of 26% and 13%, respectively).

On the basis of experimental inoculations and subsequent mortalities, Bell and Hoskins (1966) suggested that *A. viridans* might be pathogenic for the Dungeness crab, *Cancer magister*, and the shrimp, *Pandalus platyceros*, from the Pacific coast. The Pacific spiny lobster, *Panulirus interruptus*, was found subsequently to be susceptible to gaffkemia. Schapiro *et al.* (1974) increased resistance by injection of avirulent strains of the pathogen and tested a number of avirulent strains of *Aerococcus* of terrestrial origin as immunogens (H. C. Schapiro, personal communication, 1976).

Other experimental studies (Cornick and Stewart, 1968b) indicated that the bacterium may also be pathogenic for East Coast crabs (*Cancer irroratus*, *C. borealis*, and *Hyas coarctatus*). *In vitro* growth of the pathogen in crab sera was similar to that in lobster serum, suggesting susceptibility of the crabs. However, agglutinins for *A. viridans*, which were demonstrated in the sera of one of the crab species (*C. irroratus*), might counteract the favorable bacterial growth *in vivo* and reduce the severity of infections in the crab. Cornick and Stewart extended their observations by inoculations of *C. irroratus* with suspensions of the pathogen. After 49 days the surviving crabs (three) were found to be heavily infected (10^9 organisms per milliliter of hemolymph). Passage through the crabs did

not alter the pathogenicity of *Aerococcus* to lobster. A repetition of the crab inoculations with larger numbers of crabs provided some evidence for greater mortalities in experimental groups than in controls. Pathogenicity for rock crabs was less than for lobsters, as indicated by a mean time to death of 42 days in crabs, versus only 18 days in lobsters. The authors mentioned the possible role of rock crabs as reservoirs of infection for lobsters, in view of reduced pathogenicity and prolonged mean time to death in crabs.

From the foregoing it is apparent that experimental studies with *A. viridans* have been numerous and varied and have provided significant insights about the internal defenses of Crustacea. Important areas for future study include determining whether strains of the pathogen with different virulences exist and whether virulence may be increased by rapid passage through impounded lobster populations.

IV. CONCLUSIONS

Information about internal defense mechanisms in invertebrates may be summarized as follows: (1) The search for specific acquired antibodies in invertebrates has not been successful. (2) The weight of the somewhat sketchy evidence favors a major role for phagocytosis, augmented at times by relatively nonspecific innate or acquired humoral factors. (3) Many invertebrates seem endowed with antibacterial activity of body fluids, and to some extent with natural agglutinins and lysins.

Thus, many of the conclusions reached by Huff (1940) about immune mechanisms in invertebrates are still pertinent:

In the invertebrates a natural immunity to the attacks of many infective organisms and their noxious products is the first line of defense. This natural immunity is poorly understood, but it is the result of complex processes, including phagocytosis and natural antibodies, many of which are probably inherent. Phagocytosis plays a spectacular part in immunity and, as in vertebrates, is effected both by free and fixed phagocytic cells. It is ineffective in some cases. There are examples of acquired immunity—both active and passive—in invertebrates, but the specificity of such immunity is open to some question. Antibodies may be produced following inoculation or infection, but these result most successfully when natural antibodies already exist. These antibodies differ widely from the corresponding ones in vertebrates, as exemplified by the bacteriolysins which are highly thermostable. No complement has been found, but complementary actions occur. It is too early yet to attempt to evaluate the relative parts played by the various immune mechanisms in protecting the invertebrates.

Now, almost a half-century later, much additional evidence has accumulated and additional insights have been gained. The evidence suggests the existence of preformed factors which contribute to internal defense, especially the lysosomal enzymes and bactericidins. Inducible responses with some specificity include phagocyte activation and viral clearance. The hemocytes clearly perform multiple functions in addition to phagocytosis, and their biochemical role is being elucidated.

The marine invertebrates are obviously not immunologically naive; they have inhabited this hostile planet which abounds in potential pathogens too long and too successfully for that. Tiny windows on their world, often fashioned by retreaded mammalian immunologists, disclose their abilities to respond to foreign invasion—not with the specific immunoglobulin competencies of vertebrates, but with an array of non-specific, even inducible, defenses that permit survival. These defenses may be breached, however, just as they can be in vertebrates, by overwhelming infection pressure and/or by actions of environmental stressors. This is a phenomenon that we see expressed as new epizootics and consequent mass mortalities.

Stauber (1961) made the following very relevant observation:

That so few examples of acquired resistance are known among invertebrates may be quite logical. Because of the short generation time, small size, and often enormous reproductive potential of invertebrates, subsequent disease outbreaks would be much more likely to be circumvented by appearance of resistant stocks. . . . so that even with very high mortality rates, a residual stock of survivors under favorable conditions could quickly repopulate an area. . . . If this reasoning is adequate to explain the lack of evidence for acquired resistance in most invertebrates, then those with long life spans, such as *Limulus* should be studied more fully in an attempt to demonstrate acquired resistance.

Epizootics in molluscan and crustacean shellfish populations do not always follow the typical vertebrate pattern of a gradual increase in numbers of acute infections, building to a sharp peak, followed by a fairly rapid decline in percentages of acute infections, with greater persistence of chronic or latent infections. Instead, the patterns often seen in shellfish are variable in the extreme, from rapid onset followed by a rapid decline (viral gill disease of oysters in France) to the persistence of infections at high levels for long periods (*Perkinsus marinus* and *Haplosporidium nelsoni* diseases of oysters, and *krebsspest* of European crayfish). Certainly, these quantitative differences must be related in part to the internal defense mechanisms—nonspecific and of short duration in shellfish, and highly specific and anamnestic in vertebrates—as well as to the survival strategies of vertebrate versus invertebrate populations.

REFERENCES

- Aarum, G. R. (1967). Fagocytose. *Nor. Tannlaegeforenings Tid.* **77**, 243–254.
- Acton, R. T. (1970). "Immunobiological and Immunochemical Studies of the Oyster *Crassostrea virginica*," Ph.D. thesis. University of Alabama, Birmingham, Alabama.
- Acton, R. T., and Evans, E. E. (1968). Bacteriophage clearance in the oyster (*Crassostrea virginica*). *J. Bacteriol.* **95**, 1260–1266.
- Acton, R. T., Bennett, J. C., Evans, E. E., and Schrohenloher, R. E. (1969a). Physical and chemical characterization of an oyster hemagglutinin. *Biochemistry* **244**, 4128–4135.
- Acton, R. T., Weinheimer, P. F., and Evans, E. E. (1969b). A bactericidal system in the lobster *Homarus americanus*. *J. Invertebr. Pathol.* **13**, 463–464.
- Anderson, R. S. (1981). Inducible hemolytic activity in *Mercenaria mercenaria* hemolymph. *Dev. Comp. Immunol.* **5**, 575–585.
- Andrews, J. D. (1956). What killed your oysters? *South. Fisherman* **16**(7), 22–23.
- Andrews, J. D. (1964). Oyster mortality studies in Virginia. V. MSX in James River public seed beds. *Proc. Natl. Shellfish. Assoc.* **53**, 65–84.
- Andrews, J. D. (1984). Epizootiology of haplosporidan diseases affecting oysters. *Comp. Pathobiol.* **7**, 243–269.
- Andrews, J. D., and Frierman, M. (1974). Epizootiology of *Minchinia nelsoni* in susceptible wild oysters in Virginia, 1959 to 1971. *J. Invertebr. Pathol.* **24**, 127–140.
- Andrews, J. D., and Hewatt, W. G. (1957). Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. *Ecol. Monogr.* **27**, 1–26.
- Andrews, J. D., and McHugh, J. L. (1956). The survival and growth of South Carolina seed oysters in Virginia waters. *Proc. Natl. Shellfish. Assoc.* **47**, 3–17.
- Arie, B., and Stewart, J. E. (1984). Further developments in induction of resistance in *Homarus americanus* to the bacterial disease Gaffkemia. *Int. Counc. Explor. Sea, Doc. C.M.1984/F:9*, 3 pp.
- Arimoto, R., and Tripp, M. R. (1977). Characterization of a bacterial agglutinin in the hemolymph of the hard clam, *Mercenaria mercenaria*. *J. Invertebr. Pathol.* **30**, 406–413.
- Bang, F. B. (1956). A bacterial disease of *Limulus polyphemus*. *Johns Hopkins Med. J.* **98**, 325–351.
- Bang, F. B. (1961). Reaction to injury in the oyster *Crassostrea virginica*. *Biol. Bull. (Woods Hole, Mass.)* **121**, 57–68.
- Bang, F. B. (1962). Serological aspects of immunity in invertebrates. *Nature (London)* **196**, 88–89.
- Bang, F. B. (1967a). Blood clot formation in the antenna of the hermit crab *Pagurus longicarpus*. *Biol. Bull. (Woods Hole, Mass.)* **133**, 456–457.
- Bang, F. B. (1967b). Serological responses among invertebrates other than insects. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **26**, 1680–1684.
- Bang, F. B. (1968). Cellular clots in *Pagurus longicarpus* and the possible role of microtubules. *Biol. Bull. (Woods Hole, Mass.)* **135**, 414.
- Bang, F. B. (1970). Disease mechanisms in crustacean and marine arthropods. *Am. Fish. Soc., Spec. Publ.* **5**, 383–404.
- Bang, F. B. (1973). Immune reactions among marine and other invertebrates. *BioScience* **23**, 584–589.
- Bauchau, A. G., and Mengeot, J. C. (1978). Structure et fonction des hémocytes chez les crustacés. *Arch. Zool. Exp. Gén.* **119**, 227.
- Bayne, C. J. (1983). Molluscan immunobiology. In "The Mollusca" (A. S. M. Saleuddin and K. M. Wilbur, eds.), Vol. 5, Part 2, pp. 407–486. Academic Press, Orlando, Florida.

- Bayne, C. J., Sminia, T., and Van der Knaap, W. P. W. (1980). Immunological memory: Status of molluscan studies. In "Phylogeny of Immunological Memory" (M. J. Manning, ed.), pp. 57-64. Elsevier/North-Holland, Amsterdam.
- Bell, G. R. and Hoskins, G. E. (1966). Experimental transmission of the lobster pathogen, *Gaffkya homari*, to Pacific crabs and prawns. *Abstr. Annu. Meet. Can. Soc. Microbiol.*, 16th p. 21.
- Brehélin, M. (ed.) (1986). "Immunity in Invertebrates," 233 pp. Springer-Verlag, Berlin.
- Bruntz, L. (1907). Études sur les organes lymphoïdes, phagocytaires et excréteurs des crustacés supérieurs. *Arch. Zool. Exp. Gén.* 7, 1-67.
- Cantacuzène, J. (1913). Sur la production d'anticorps artificiels chez *Eupagurus prideauxii*. *C. R. Séances Soc. Biol.* 74, 293-295.
- Cantacuzène, J. (1921). Quelques remarques au sujet d'une infection expérimentale chez *Maia squinado*. *C. R. Séances Soc. Biol.* 84, 1007-1009.
- Cantacuzène, J. (1923). Le problème de l'immunité chez les invertébrés. *C. R. Séances Soc. Biol.* 88(Suppl.), 48-119.
- Cantacuzène, J. (1925). Immunité d'*Eupagurus prideauxii* vis-à-vis des poisons de l'*Adamsia palliata*. *C. R. Séances Soc. Biol.* 92, 1133-1136.
- Cantacuzène, J., and Damboviceanu, A. (1934a). Caractères biologiques de l'extrait des accoties d'*Adamsia palliata* après déprotéinisation. *C. R. Séances Soc. Biol.* 117, 136-138.
- Cantacuzène, J., and Damboviceanu, A. (1934b). Caractères physico-chimiques du poison des accoties d'*Adamsia palliata*. *C. R. Séances Soc. Biol.* 117, 138-140.
- Cattaneo, G. (1888). Sugli "amebociti" dei crustacei. *Zool. Anz.* 11, 452-455.
- Cheng, T. C. (1967). Marine molluscs as hosts for symbioses: With a review of known parasites of commercially important species. *Adv. Mar. Biol.* 5, 1-424.
- Cheng, T. C. (1981). Bivalves. In "Invertebrate Blood Cells" (N. A. Ratcliffe and A. F. Rowley, eds.), Academic Press, London.
- Cheng, T. C. (1983a). The role of lysosomes in molluscan inflammation. *Am. Zool.* 23, 129-144.
- Cheng, T. C. (1983b). Triggering of immunologic defense mechanisms of molluscan shellfish by biotic and abiotic challenge and its applications. *Mar. Technol. Soc. J.* 17, 18-25.
- Cheng, T. C., and Rifkin, E. (1970). Cellular reactions in marine molluscs in response to helminth parasitism. *Am. Fish. Soc., Spec. Publ.* 5, 443-496.
- Cheng, T. C., and Rodrick, G. E. (1974). Identification and characterization of lysozyme from the hemolymph of the soft-shelled clam, *Mya arenaria*. *Biol. Bull. (Woods Hole, Mass.)* 147, 311-320.
- Cheng, T. C., and Rodrick, G. E. (1975). Lysosomal and other enzymes in the hemolymph of *Crassostrea virginica* and *Mercenaria mercenaria*. *Comp. Biochem. Physiol. B.* 52, 443-447.
- Cheng, T. C., and Yoshino, T. P. (1975). Lipase activity in the serum and hemolymph cells of the soft-shelled clam, *Mya arenaria*, during phagocytosis. *J. Invertebr. Pathol.* 27, 243-245.
- Cheng, T. C., Rodrick, G. E., Foley, D. A., and Koehler, S. A. (1975). Release of lysozyme from hemolymph cells of *Mercenaria mercenaria* during phagocytosis. *J. Invertebr. Pathol.* 25, 261-265.
- Cohen, W. D., Nemhauser, I., and Jaeger, R. (1977). Rapid visualization of the marginal band system in blood cells of marine species. *Biol. Bull. (Woods Hole, Mass.)* 153, 420.
- Cohen, W. D., Nemhauser, I., and Cohen, M. F. (1983). Marginal bands of lobster blood cells: Disappearance associated with changes in cell morphology. *Biol. Bull. (Woods Hole, Mass.)* 164, 50-61.
- Cohn, Z. A., Hirsch, J. G., and Wiener, E. (1963). The cytoplasmic granules of phagocytic cells and the degradation of bacteria. In "Lysosomes" (A. V. S. de Reuck and M. P. Cameron, eds.), pp. 126-144. Little, Brown, Boston.

- Cooper, E. L. (1976). "Comparative Immunology," 338 pp. Prentice-Hall, Englewood Cliffs, New Jersey.
- Cornick, J. W., and Stewart, J. E. (1968a). Interaction of the pathogen *Gaffkya homari* with natural defense mechanisms of *Homarus americanus*. *J. Fish. Res. Board Can.* **25**, 695-709.
- Cornick, J. W., and Stewart, J. E. (1968b). Pathogenicity of *Gaffkya homari* for the crab *Cancer irroratus*. *J. Fish. Res. Board Can.* **25**, 795-799.
- Cornick, J. W., and Stewart, J. E. (1973). Partial characterization of a natural agglutinin in the hemolymph of the lobster, *Homarus americanus*. *J. Invertebr. Pathol.* **21**, 255-262.
- Cuénot, L. (1895). Études physiologiques sur les crustacés décapodes. *Arch. Biol. Liege* **8**, 245-303.
- Cuénot, L. (1897). Les globules sanguins et les organes lymphoïdes des invertébrés. (revue critique et nouvelles recherches). *Arch. Anat. Microsc. Morphol. Exp.* **1**, 153-192.
- Cuénot, L. (1898). Études physiologiques sur les oligochètes. *Arch. Biol. Liege* **15**, 114-124.
- Cuénot, L. (1905). L'organe phagocytaire des crustacés décapodes. *Arch. Zool. Exp. Gén., Ser. 4* **3**, 1-15.
- Cushing, J. E. (1967). Invertebrates, immunology and evolution. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **26**, 1666-1670.
- Cushing, J. E., Calaprice, N. L., and Trump, G. (1963). Blood group reactive substances in some marine invertebrates. *Biol. Bull. (Woods Hole, Mass.)* **125**, 69-80.
- Cushing, J. E., Evans, E. E., and Evans, M. L. (1971). Induced bactericidal responses of abalones. *J. Invertebr. Pathol.* **17**, 446-448.
- Douglass, W. R. (1977). "Minchinia nelsoni Disease Development, Host Defense Reactions, and Hemolymph Enzyme Alterations in Stocks of Oysters (*Crassostrea virginica*) Resistant and Susceptible to *Minchinia nelsoni*-Caused Mortality," Ph.D. dissertation. Rutgers University, New Brunswick, New Jersey.
- Douglass, W. R., and Haskin, H. H. (1976). Oyster-MSX interactions: Alterations in hemolymph enzyme activities in *Crassostrea virginica* during the course of *Minchinia nelsoni* disease development. *J. Invertebr. Pathol.* **27**, 317-323.
- Durliat, M. (1985). Clotting processes in Crustacea Decapoda. *Biol. Rev.* **60**, 473-489.
- Durliat, M., and Vranckx, R. (1981). Action of various anticoagulants on hemolymphs of lobsters and spiny lobsters. *Biol. Bull. (Woods Hole, Mass.)* **160**, 55-68.
- Durliat, M., and Vranckx, R. (1982a). Proteins of aqueous extracts from the hepatopancreas of *Astacus leptodactylus*. 1. Changes in proteins during the molt cycle. *Comp. Biochem. Physiol. B* **71**, 155-163.
- Durliat, M., and Vranckx, R. (1982b). Proteins of aqueous extracts from the hepatopancreas of *Astacus leptodactylus*. 2. Immunological identities of proteins from hepatopancreas and blood. *Comp. Biochem. Physiol. B* **71**, 165-171.
- Evans, E. E., Painter, B., Evans, M. L., Weinheimer, P., and Acton, R. T. (1968). An induced bactericidin in the spiny lobster, *Panulirus argus*. *Proc. Soc. Exp. Biol. Med.* **128**, 394-398.
- Evans, E. E., Cushing, J. E., Sawyer, S., Weinheimer, P. F., Acton, R. T., and McNeely, J. L. (1969a). Induced bactericidal response in the spiny lobster *Panulirus interruptus*. *Proc. Soc. Exp. Biol. Med.* **132**, 111-114.
- Evans, E. E., Weinheimer, P. F., Painter, B., Acton, R. T., and Evans, M. L. (1969b). Secondary and tertiary responses of the induced bactericidin from the West Indian spiny lobster, *Panulirus argus*. *J. Bacteriol.* **98**, 943-946.
- Farley, C. A. (1968). *Minchinia nelsoni* (Haplosporida) disease syndrome in the American oyster *Crassostrea virginica*. *J. Protozool.* **15**, 585-599.
- Farley, C. A. (1975). Epizootic and enzootic aspects of *Minchinia nelsoni* (Haplosporida) disease in Maryland oysters. *J. Protozool.* **22**, 418-427.

- Feng, S. Y. (1967). Responses of molluscs to foreign bodies, with special reference to the oyster. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **26**, 1685–1692.
- Feng, S. Y., and Canzonier, W. J. (1970). Humoral responses in the American oyster (*Crassostrea virginica*) infected with *Bucephalus* sp. and *Minchinia nelsoni*. *Am. Fish. Soc., Spec. Publ.* **5**, 497–510.
- Feng, S. Y., Khairallah, E. A., and Canzonier, W. J. (1970). Hemolymph-free amino acids and related nitrogenous compounds of *Crassostrea virginica* infected with *Bucephalus* sp. and *Minchinia nelsoni*. *Comp. Biochem. Physiol.* **34**, 547–556.
- Florkin, M. (1960). Blood chemistry. In "The Physiology of Crustacea" (T. H. Waterman, ed.), Vol. I, pp. 141–159. Academic Press, New York.
- Foley, D. A., and Cheng, T. C. (1975). A quantitative study of phagocytosis by hemolymph cells of the pelecypods *Crassostrea virginica* and *Mercenaria mercenaria*. *J. Invertebr. Pathol.* **25**, 189–197.
- Fontaine, C. T., and Lightner, D. V. (1975). Cellular response to injury in penaeid shrimp. *Mar. Fish. Rev.* **37**(5–6), 4–10.
- Ford, S. E. (1985a). Chronic infections of *Haplosporidium nelsoni* (MSX) in the oyster *Crassostrea virginica*. *J. Invertebr. Pathol.* **45**, 94–107.
- Ford, S. E. (1985b). Effects of salinity on survival of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber, and Mackin) in oysters. *J. Shellfish Res.* **5**, 85–90.
- Ford, S. E. (1986). Comparison of hemolymph proteins from resistant and susceptible oysters, *Crassostrea virginica*, exposed to the parasite *Haplosporidium nelsoni* (MSX). *J. Invertebr. Pathol.* **47**, 283–294.
- Ford, S. E. (1988). Host–parasite interactions in eastern oysters selected for resistance to *Haplosporidium nelsoni* (MSX) disease: Survival mechanisms against a natural pathogen. *Am. Fish. Soc., Spec. Publ.* **18**, 206–224.
- Ford, S. E., and Haskin, H. H. (1982). History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957–1980. *J. Invertebr. Pathol.* **40**, 118–141.
- Ford, S. E., and Haskin, H. H. (1987). Infection and mortality patterns in strains of oysters *Crassostrea virginica* selected for resistance to the parasite *Haplosporidium nelsoni* (MSX). *J. Parasitol.* **73**, 368–376.
- Fredericq, H. (1879). Note sur le sang de l'homard. *Bull. Cl. Sci., Acad. R. Belg., Ser. 2* **47**, 409–413.
- Fries, C. R. (1985). Protein hemolymph factors and their roles in invertebrate defense mechanisms: A review. *Comp. Pathol.* **6**, 50–86.
- George, W. C., and Nichols, J. (1948). A study of the blood of some Crustacea. *J. Morphol.* **83**, 425–443.
- Gibson, F. A. (1961). Gaffkaemia in stored lobsters. *Int. Counc. Explor. Sea, Shellfish Comm. Doc.* **58**, 1 p.
- Goggins, P. L., and Hurst, J. W., Jr. (1960). Progress report on lobster gaffkyaremia (red tail). *Bull. Maine Dep. Sea Shore Fish.* 9 pp. (mimeo).
- Goldenberg, P. Z., and Greenberg, A. H. (1983). Functional heterogeneity of carbohydrate-binding hemolymph proteins: Evidence of a nonagglutinating opsonin in *Homarus americanus*. *J. Invertebr. Pathol.* **42**, 33–41.
- Goldenberg, P. Z., Huebner, E., and Greenberg, A. H. (1984). Activation of lobster hemocytes for phagocytosis. *J. Invertebr. Pathol.* **43**, 77–88.
- Häll, L., and Söderhäll, K. (1982). Purification and properties of a protease inhibitor from crayfish hemolymph. *J. Invertebr. Pathol.* **39**, 29–37.
- Halliburton, W. E. (1885). On the blood of the decapod Crustacea. *J. Physiol. (London)* **6**, 300–335.

- Hardy, W. B. (1892). The blood corpuscles of the Crustacea, together with a suggestion as to the origin of the crustacean fibrin-ferment. *J. Physiol. (London)* **13**, 165–190.
- Hardy, S. W., Fletcher, T. C., and Olafsen, J. A. (1977). Aspects of cellular and humoral defence mechanisms in the Pacific oyster, *Crassostrea gigas*. In "Developmental Immunobiology" (J. B. Solomon and J. D. Horton, eds.), pp. 59–66. Elsevier/North-Holland, Amsterdam.
- Haskin, H. H., and Douglass, W. R. (1971). Experimental approaches to oyster-MSX interactions. *Proc. Natl. Shellfish. Assoc.* **61**, 4.
- Haskin, H. H., and Ford, S. E. (1979). Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. *Mar. Fish. Rev.* **41**, 54–63.
- Haskin, H. H., and Ford, S. E. (1982). *Haplosporidium nelsoni* (MSX) on Delaware Bay seed oyster beds: A host-parasite relationship along a salinity gradient. *J. Invertebr. Pathol.* **40**, 388–405.
- Haskin, H. H., and Ford, S. E. (1987). Breeding for disease resistance in molluscs. In "Proceedings of a World Symposium on Selection, Hybridization, and Genetic Engineering in Aquaculture" (K. Tiews, ed.), Vol. 2, pp. 431–441. Bordeaux, France.
- Haskin, H. H., Canzonier, W. J., and Myhre, J. L. (1965). The history of "MSX" on Delaware Bay oyster grounds 1957–65. *Am. Malacol. Union Bull.* **32**, 20–21.
- Hergenbahn, H. G., and Söderhäll, K. (1985). α_2 -Macroglobulin like activity in plasma of the crayfish, *Pacifastacus leniusculus*. *Comp. Biochem. Physiol. B* **81**, 833–835.
- Hirsch, J. G. (1959). Immunity to infectious diseases: Review of some concepts of Metchnikoff. *Bacteriol. Rev.* **23**, 48–60.
- Hirsch, J. G. (1965). Phagocytosis. *Annu. Rev. Microbiol.* **19**, 339–350.
- Hitchner, E. R., and Snieszko, S. F. (1947). A study of a microorganism causing a bacterial disease of lobsters. *J. Bacteriol.* **54**, 48 (abstract).
- Huff, C. G. (1940). Immunity in invertebrates. *Physiol. Rev.* **20**, 68–88.
- Johansson, M. W., and Söderhäll, K. (1985). Exocytosis of the prophenoloxidase activating system from crayfish haemocytes. *J. Comp. Physiol. B* **156**, 175–181.
- Johnson, H. M. (1964). Human blood group A specific agglutinin of the butter clam, *Saxidomus giganteus*. *Science* **146**, 548.
- Johnson, P. T. (1976). Bacterial infection in the blue crab, *Callinectes sapidus*: Course of infection and histopathology. *J. Invertebr. Pathol.* **28**, 25–36.
- Johnson, P. T. (1987). A review of fixed phagocytic and pinocytotic cells of decapod crustaceans, with remarks on hemocytes. *Dev. Comp. Immunol.* **11**, 679–704.
- Kimber, I. (1985). Natural killer cells. *Med. Lab. Sci.* **42**, 60–63.
- Kinne, O. (Ed.) (1980). "Diseases of Marine Animals," Vol. I, 466 pp. Biol. Anstalt Helgol., Hamburg, FRG.
- Lane, C. E., Coursen, B. W., and Hines, K. (1961). Biologically active peptides in *Physalia* toxin. *Proc. Soc. Exp. Biol. Med.* **107**, 670–672.
- Leippe, M., and Renwantz, L. (1988). Release of cytotoxic and agglutinating molecules by *Mytilus* hemocytes. *Dev. Comp. Immunol.* **12**, 297–308.
- Levin, J. (1967). Blood coagulation and endotoxin in invertebrates. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **26**, 1707–1712.
- Logie, R. R. (1956). Oyster mortalities, old and new, in the Maritimes. *Prog. Rep. Atl. Coast Stn.* **65**, 3–11.
- Logie, R. R., Drinnan, R. E., and Henderson, E. B. (1960). Rehabilitation of disease-depleted oyster populations in eastern Canada. *Proc. Annu. Sess. Gulf Caribb. Fish. Inst.* **13**, 109–113.
- Löwit, M. (1889). "Über die Beziehungen der weissen Blutkörperchen zur Blutgerinnung. *Ziegler's Beitr. Pathol. Anat.* **5**, 469–520.

- Mackin, J. G. (1951). Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, and Collier. *Bull. Mar. Sci. Gulf Caribb.* **1**, 72–87.
- Mackin, J. G. (1956). *Dermocystidium marinum* and salinity. *Proc. Natl. Shellfish. Assoc.* **46**, 116–128.
- Mackin, J. G. (1962). Oyster disease caused by *Dermocystidium marinum* and other micro-organisms in Louisiana. *Publ. Inst. Mar. Sci., Univ. Tex.* **7**, 132–229.
- Mackin, J. G., Korrinda, P., and Hopkins, S. H. (1952). Hexamitiasis of *Ostrea edulis* L. and *Crassostrea virginica* (Gmelin). *Bull. Mar. Sci. Gulf Caribb.* **1**, 266–277.
- Madaras, F., Parkin, J. D., and Castaldi, P. A. (1979). Coagulation in the sand crab (*Ovalipes bipustulatus*). *Thromb. Haemostasis* **42**, 734–742.
- Madaras, F., Chew, M. Y., and Parkin, J. D. (1981). Purification and characterization of the sand crab (*Ovalipes bipustulatus*) coagulogen (fibrinogen). *Thromb. Haemostasis* **45**, 77–81.
- Manning, M. J., and Turner, R. J. (1976). "Comparative Immunobiology." Wiley, New York.
- Maramarosch, K., and Shope, R. E. (Eds.) (1975). "Invertebrate Immunity: Mechanisms of Invertebrate Vector-Parasite Relations," 365 pp. Academic Press, New York.
- Marx, J. L. (1986). How killer cells kill their targets. *Science* **231**, 1367–1369.
- Maynard, D. M. (1960). Circulation and heart function. In "The Physiology of Crustacea" (T. H. Waterman, ed.), Vol. I pp. 161–226. Academic Press, New York.
- McCumber, L. J., and Clem, L. W. (1977). Recognition of viruses and xenogenic proteins by the blue crab, *Callinectes sapidus*: I. Clearance and organ concentration. *Dev. Comp. Immunol.* **1**, 5–14.
- McCumber, L. J., Hoffmann, E. M., and Clem, L. W. (1979). Recognition of viruses and xenogenic proteins by the blue crab, *Callinectes sapidus*: A humoral receptor for T₂ bacteriophage. *J. Invertebr. Pathol.* **33**, 1–9.
- McDade, J. E., and Tripp, M. R. (1967a). Lysozyme in the hemolymph of the oyster, *Crassostrea virginica*. *J. Invertebr. Pathol.* **9**, 531–535.
- McDade, J. E., and Tripp, M. R. (1967b). Lysozyme in oyster mantle mucus. *J. Invertebr. Pathol.* **9**, 581–582.
- McKay, D., and Jenkin, C. R. (1969). Immunity in the invertebrates. II. Adaptive immunity in the crayfish (*Parachanna bicarinatus*). *Immunology* **17**, 127–137.
- McKay, D., and Jenkin, C. R. (1970a). Immunity of invertebrates. The role of serum factors in the phagocytosis of erythrocytes by haemocytes of the freshwater crayfish (*Parachanna bicarinatus*). *Aust. J. Exp. Biol. Med. Sci.* **48**, 139–150.
- McKay, D., and Jenkin, C. R. (1970b). Immunity in the invertebrates. Correlation of the phagocytic activity of haemocytes with resistance to infection in the crayfish (*Parachanna bicarinatus*). *Aust. J. Exp. Biol. Med. Sci.* **48**, 609–617.
- McKay, D., Jenkin, C. R., and Rowley, D. (1969). Immunity in the invertebrates. I. Studies on the naturally occurring haemagglutinins in the fluid from invertebrates. *Aust. J. Exp. Biol. Med. Sci.* **47**, 125–134.
- Mengebier, W. L., and Wood, L. (1969). The effects of *Minchinia nelsoni* infection on enzyme levels in *Crassostrea virginica*. II. Serum phosphohexose isomerase. *Comp. Biochem. Physiol.* **29**, 265–270.
- Mengeot, J. C., Bauchau, A. G., de Brouwer, M. B., and Passelecq-Gérin, E. (1976). Séparation des granules présents dans les hémocytes des crustacés par exocytose provoquée. *Comp. Biochem. Physiol. A* **54**, 145–148.
- Mengeot, J. C., Bauchau, A. G., De Brouwer, M. B., and Passelecq-Gérin, E. (1977). Isolement des granules des hémocytes de *Homarus vulgaris*. Examens électrophorétiques du contenu protéique des granules. *Comp. Biochem. Physiol. A* **58**, 393–403.
- Metchnikoff, E. (1884). Ueber eine Sprosspilzkrankheit der Daphnien. *Arch. Pathol. Anat.* **96**, 177–195.

- Miles, A. A. (1962). Mechanisms of immunity in bacterial infections. *Recent Prog. Microbiol.* **7**, 399–409.
- Mori, K., and Stewart, J. E. (1978a). The hemolymph bactericidin of American lobster (*Homarus americanus*): Adsorption and activation. *J. Fish. Res. Board Can.* **35**, 1504–1507.
- Mori, K., and Stewart, J. E. (1978b). Natural and induced bactericidal activities of the hepatopancreas of the American lobster, *Homarus americanus*. *J. Invertebr. Pathol.* **32**, 171–176.
- Mori, K., Tone, Y., Suzuki, T., Kasahara, K., and Nomura, T. (1980a). Defense mechanisms of molluscs—I. Bactericidal and agglutinin activities in the scallop tissues. *Nippon Suisan Gakkaishi* **46**, 717–722.
- Mori, K., Itsukaichi, S., Murayama, K., and Nomura, T. (1980b). Activities of agglutinin and bactericidin in oyster tissues. *Nippon Suisan Gakkaishi* **46**, 1385–1389.
- Mori, K., Murayama, K., Kanno, N., Nakamura, M., Ohira, E., Kato, Y., and Nomura, T. (1984). Occurrence and characterization of the defense-factors in the Japanese oyster *Crassostrea gigas*. *Tohoku J. Agric. Res.* **35**, 55–68.
- Myhre, J. L. (1973). "Levels of Infection in Spat of *Crassostrea virginica* and Mechanisms of Resistance to the Haplosporidan Parasite *Minchinia nelsoni*". M.A. thesis. Rutgers University, New Brunswick, New Jersey.
- Myhre, J. L., and Haskin, H. H. (1968). Some observations on the development of early *Minchinia nelsoni* infections in *Crassostrea virginica* and some aspects of the host-parasite relationship. *Proc. Natl. Shellfish. Assoc.* **58**, 6.
- Myhre, J. L., and Haskin, H. H. (1970). MSX infections in resistant and susceptible oyster stocks. *Proc. Natl. Shellfish. Assoc.* **60**, 9.
- Nakamura, M., Mori, K., Inooka, S., and Nomura, T. (1985). *In vitro* production of hydrogen peroxide by the amoebocytes of the scallop *Patinopecten yessoensis* (Jay). *Dev. Comp. Immunol.* **9**, 407–417.
- Needler, A. W. H. (1931). The oysters of Malpeque Bay. *Bull. Biol. Board Can.* **22**, 1–30.
- Needler, A. W. H., and Logie, R. R. (1947). Serious mortalities in Prince Edward Island oysters caused by a contagious disease. *Trans. R. Soc. Can., Ser. 3, Sect. 5* **41**, 73–89.
- Nelstrup, A. E., Taylor, G., and Collard, P. (1968). Studies on phagocytosis. III. Antigen clearance studies in invertebrates and poikilothermic vertebrates. *Immunology* **14**, 347–356.
- Newman, M. C., and Feng, S. Y. (1982). Susceptibility and resistance of the rock crab, *Cancer irroratus*, to natural and experimental bacterial infection. *J. Invertebr. Pathol.* **40**, 75–88.
- Osawa, S., and Yabuubhi, E. (1963). Anticorps produits par le homard americain, *Cambarus clarkii*. *C. R. Séances Soc. Biol.* **156**, 698–699.
- Otto, S. V., and Krantz, G. E. (1977). An epizootic of "dermo" disease in oysters in the Maryland portion of the Chesapeake Bay. *Proc. Natl. Shellfish. Assoc.* **67**, 121.
- Parry, G. (1960). Excretion. In "The Physiology of Crustacea" Vol. I, (T. H. Waterman, ed.), pp. 341–366. Academic Press, New York.
- Paterson, W. D., and Stewart, J. E. (1979). Rate and duration of phagocytic increase in lobsters induced by *Pseudomonas perolens* endotoxin. *Dev. Comp. Immunol.* **3**, 353–357.
- Paterson, W. D., Stewart, J. E., and Zwicker, B. M. (1976). Phagocytosis as a cellular immune response mechanism in the American lobster *Homarus americanus*. *J. Invertebr. Pathol.* **27**, 95.
- Paterson, W. D., Keith, I., Airdrie, D., and Boston, L. (1988). A commercial vaccine to prevent gaffkemia in lobsters: Development and performance. *Prog. Abstr. Annu. Meet. Soc. Invertebr. Pathol.*, 21st p. 43 (abstract).

- Pixell-Goodrich, H. L. (1928). Reactions of *Gammarus* to injury and disease with notes on microsporidial and fungoid diseases. *J. Cell Sci.* **72**, 325–353.
- Poisson, R. (1930). Observations sur *Anophrys sarcophaga* Cohn (= *A. maggii* Cattaneo). *Bull. Biol. Fr. Belg.* **64**, 288–331.
- Rabin, H. (1965). Studies on gaffkemia, a bacterial disease of the American lobster, *Homarus americanus* (Milne-Edwards). *J. Invertebr. Pathol.* **7**, 391–397.
- Rabin, H., and Hughes, J. T. (1968). Studies on host–parasite relationships in gaffkemia. *J. Invertebr. Pathol.* **10**, 335–344.
- Ratcliffe, N. A., Rowley, A. F., Fitzgerald, S. W., and Rhodes, C. P. (1985). Invertebrate immunity: Basic concepts and recent advances. *Int. Rev. Cytol.* **97**, 183–350.
- Ray, S. M. (1954). Biological studies of *Dermocystidium marinum*, a fungus parasite of oysters. *Rice Inst. Pam., Spec. Issue*, 114 pp.
- Read, C. P. (1958). Status of behavioral and physiological “resistance.” *Rice Inst. Pam.* **45**, 36–54.
- Renwrantz, L., and Stahmer, A. (1983). Opsonizing properties of an isolated hemolymph agglutinin and demonstration of lectin-like recognition molecules at the surface of hemocytes from *Mytilus edulis*. *J. Comp. Physiol. B* **149**, 535–547.
- Renwrantz, L., Schänke, W., Harm, H., Erl, H., Liebsch, H., and Gercken, J. (1981). Discriminative ability and function of the immunobiological recognition system of the snail *Helix pomatia*. *J. Comp. Physiol.* **141**, 477–488.
- Robineaux, J., and Frederic, J. (1955). Contribution à l’étude des granulations neutrophiles des polynucléaires par la microcinématographie en contraste de phase. *C. R. Séances Soc. Biol.* **149**, 486–489.
- Rodrick, G. E., and Cheng, T. C. (1974). Kinetic properties of lysozyme from *Crassostrea virginica* hemolymph. *J. Invertebr. Pathol.* **24**, 41–48.
- Roskam, R. (1957). Gaffkaemia, a contagious disease in *Homarus vulgaris*. *Int. Counc. Explor. Sea, Shellfish Comm. Doc.* 4 pp. (mimeo).
- Rowley, D. (1960). Antibacterial systems of serum in relation to non-specific immunity to infection. *Bacteriol. Rev.* **24**, 106–114.
- Sanderson, C. J. (1981). The mechanism of lymphocyte mediated cytotoxicity. *Biol. Rev.* **56**, 153–197.
- Schapiro, H. C. (1975). Immunity in decapod crustaceans. *Am. Zool.* **15**, 13–19.
- Schapiro, H. C., Mathewson, J. H., Steenbergen, J. F., Kellogg, S., Ingram, C., Nierengarten, G., and Rabin, H. (1974). Gaffkemia in the California spiny lobster, *Panulirus interruptus*: Infection and immunization. *Aquaculture* **3**, 403–408.
- Schneider, T. A. (1951). Nutrition and resistance–susceptibility to infection. *Am. J. Trop. Med. Hyg.* **31**, 174–182.
- Sindermann, C. J. (1962). Serology of Atlantic clupeoid fishes. *Am. Nat.* **96**, 225–231.
- Sindermann, C. J. (1963). Use of plant hemagglutinins in serological studies of clupeoid fishes. *U.S. Fish Wildl. Serv., Fish. Bull.* **63**, 137–141.
- Sindermann, C. J. (1971). Internal defenses of Crustacea: A review. *Natl. Mar. Fish. Serv., Fish. Bull.* **69**, 455–489.
- Sindermann, C. J., and Mairs, D. F. (1959). A major blood group system in Atlantic sea herring. *Copeia* **1959**, 228–232.
- Sminia, T., and van der Knapp, W. P. W. (1987). Cells and molecules in molluscan immunology. *Dev. Comp. Immunol.* **11**, 17–28.
- Snieszko, S. F., and Taylor, C. C. (1947). A bacterial disease of the lobster, *Homarus americanus*. *Science* **105**, 500.
- Söderhäll, K. (1982). Prophenoloxidase activating system and melanization—A recognition mechanism of arthropods? A review. *Dev. Comp. Immunol.* **6**, 601–611.

- Söderhäll, K. (1983). β 1,3-Glucan enhancement of protease activity in crayfish haemocyte lysate. *Comp. Biochem. Physiol. B* **74**, 221–224.
- Söderhäll, K. (1986). The cellular immune system in crustaceans. *Proc. Int. Colloq. Invertebr. Pathol.*, 4th pp. 417–420.
- Söderhäll, K., and Ajaxon, R. (1982). Effect of quinones and melanin on mycelial growth of *Aphanomyces* spp. and extracellular protease of *Aphanomyces astaci* parasite on crayfish. *J. Invertebr. Pathol.* **39**, 105–109.
- Söderhäll, K., and Häll, L. (1984). Lipopolysaccharide induced activation of prophenoloxidase activating system in crayfish haemocyte lysate. *Biochim. Biophys. Acta* **797**, 99–104.
- Söderhäll, K., and Smith, V. J. (1983). Separation of the haemocyte populations of *Carcinus maenas* and other marine decapods and prophenoloxidase distribution. *Dev. Comp. Immunol.* **7**, 229–239.
- Söderhäll, K., and Smith, V. J. (1986). The prophenoloxidase activating system: The biochemistry of its activation and role in arthropod cellular immunity with special reference to crustaceans. In "Immunity in Invertebrates" (M. Brehélin, ed.), pp. 208–223. Springer-Verlag, Berlin.
- Söderhäll, K., Wingren, A., Johansson, M. W., and Bertheussen, K. (1985). The cytotoxic reaction of hemocytes from the freshwater crayfish, *Astacus astacus*. *Cell. Immunol.* **94**, 326–330.
- Solangi, M. A., and Lightner, D. V. (1976). Cellular inflammatory response of *Penaeus aztecus* and *P. setiferus* to the pathogenic fungus, *Fusarium* sp., isolated from the California brown shrimp, *P. californiensis*. *J. Invertebr. Pathol.* **27**, 77–86.
- Solomon, J. B., and Horton, J. D. (eds.) (1977). "Developmental Immunobiology." Elsevier/North-Holland, Amsterdam.
- Sprague, V., Dunnington, E. A., and Drobeck, E. (1969). Decrease in incidence of *Minchinia nelsoni* in oysters accompanying reduction of salinity in the laboratory. *Proc. Natl. Shellfish. Assoc.* **59**, 23–26.
- Stauber, L. A. (1950). The fate of India ink injected intracardially into the oyster, *Ostrea virginica* Gmelin. *Biol. Bull. (Woods Hole, Mass.)* **89**, 227–241.
- Stauber, L. A. (1961). Immunity in invertebrates with special reference to the oyster. *Proc. Natl. Shellfish. Assoc.* **50**, 7–20.
- Stewart, J. E. (1975). Gaffkemia, the fatal infection of lobsters (genus *Homarus*) caused by *Aerococcus viridans* (var.) *homari*: A review. *Mar. Fish. Rev.* **37**(5–6), 20–24.
- Stewart, J. E. (1980). Diseases. In "The Biology and Management of Lobsters" (J. S. Cobb, and B. F. Philipps, eds.), pp. 301–342. Academic Press, New York.
- Stewart, J. E. (1984). Lobster diseases. *Helgol. Wiss. Meeresunters.* **37**, 243–254.
- Stewart, J. E., and Arie, B. (1974). Effectiveness of vancomycin against gaffkemia, the bacterial disease of lobsters (genus *Homarus*). *J. Fish. Res. Board Can.* **31**, 1873–1879.
- Stewart, J. E., and Foley, D. M. (1969). A precipitin reaction of the hemolymph of the lobster *Homarus americanus*. *J. Fish. Res. Board Can.* **26**, 1392–1397.
- Stewart, J. E., and Rabin, H. (1970). Gaffkemia, a bacterial disease of lobsters (genus *Homarus*). *Am. Fish. Soc., Spec. Publ.* **5**, 431–439.
- Stewart, J. E., and Zwicker, B. M. (1972). Natural and induced bactericidal activities of the lobster, *Homarus americanus* products of hemocyte–plasmas interaction. *Can. J. Microbiol.* **18**, 1499–1509.
- Stewart, J. E., and Zwicker, B. M. (1974a). A comparison of various vaccines for inducing resistance in the lobster (*Homarus americanus*) to the bacterial infection, gaffkemia. *J. Fish. Res. Board Can.* **31**, 1887–1892.
- Stewart, J. E., and Zwicker, B. M. (1974b). Induction of internal defense mechanisms in the lobster *Homarus americanus*. *Contemp. Top. Immunobiol.* **4**, 233–239.

- Stewart, J. E., Dingle, J. R., and Odense, P. H. (1966). Constituents of the hemolymph of the lobster, *Homarus americanus* Milne-Edwards. *Can. J. Biochem.* **44**, 1447–1459.
- Stewart, J. E., Arie, B., Zwicker, B. M., and Dingle, J. R. (1969a). Gaffkemia, a bacterial disease of the lobster, *Homarus americanus*: Effects of the pathogen *Gaffkya homari*, on the physiology of the host. *Can. J. Microbiol.* **15**, 925–932.
- Stewart, J. E., Dockrill, A., and Cornick, J. W. (1969b). Influence of temperature on gaffkemia, a bacterial disease of the lobster, *Homarus americanus*. *J. Fish. Res. Board Can.* **26**, 2503–2510.
- Stewart, J. E., Foley, D. M., and Ackman, R. G. (1969c). Characteristics of *Gaffkya homari* the causative agent of the lobster disease, gaffkemia. *J. Fish. Res. Board Can.* **26**, 1385–1389.
- Suter, E. (1956). Interaction between phagocytes and pathogenic microorganisms. *Bacteriol. Rev.* **20**, 94–132.
- Tait, J. (1918a). Capillary phenomena observed in blood cells: Thigmocytes, phagocytosis, amoeboid movement, differential adhesiveness of corpuscles, emigration of leucocytes. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **17**, 1–33.
- Tait, J. (1918b). The blood of *Astacus*. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **8**, 1–40.
- Tait, J., and Gunn, J. D. (1918). The blood of *Astacus fluviatilis*: A study in crustacean blood with special reference to coagulation and phagocytosis. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **12**, 35–80.
- Taylor, R. L. (1969). A suggested role for the polyphenol-phenoloxidase system in invertebrate immunity. *J. Invertebr. Pathol.* **14**, 427–428.
- Taylor, A. E., Taylor, G., and Collard, P. (1964). Secondary immune response to bacteriophage T₁ in the shore crab, *Carcinus maenas*. *Nature (London)* **203**, 775.
- Teague, P. O., and Friou, G. J. (1964). Lack of immunological responses by an invertebrate. *Comp. Biochem. Physiol.* **12**, 471–478.
- Tripp, M. R. (1960). Mechanisms of removal of injected microorganisms from the American oyster, *Crassostrea virginica* (Gmelin). *Biol. Bull. (Woods Hole, Mass.)* **119**, 210–223.
- Tripp, M. R. (1963). Cellular responses of mollusks. *Ann. N.Y. Acad. Sci.* **113**, 467–474.
- Tripp, M. R. (1966). Hemagglutinin in the blood of the oyster *Crassostrea virginica*. *J. Invertebr. Pathol.* **8**, 478–484.
- Tripp, M. R. (1974). Oyster hemolymph proteins. *Ann. N. Y. Acad. Sci.* **234**, 18–33.
- Tripp, M. R. (1975). Humoral factors and molluscan immunity. In "Invertebrate Immunity: Mechanisms of Invertebrate Vector-Parasite Relations" (K. Maramorosch and R. E. Shope, eds.), pp. 201–223. Academic Press, New York.
- Tsujii, T. (1960). Studies on the mechanism of shell- and pearl-formation in Mollusca. *Mie-Kenritsu Daigaku Suisangakubu Kiyo* **5**, 1–70.
- Tyler, A. (1946). Natural haemagglutinins in the body fluids and seminal fluids of various invertebrates. *Biol. Bull. (Woods Hole, Mass.)* **90**, 213.
- Tyler, A., and Metz, C. B. (1945). Natural heteroagglutinins in the serum of the spiny lobster, *Panulirus interruptus*. I. Taxonomic range of activity, electrophoretic and immunizing properties. *J. Exp. Zool.* **100**, 387–406.
- Tyler, A., and Scheer, B. T. (1945). Natural heteroagglutinins in the serum of the spiny lobster, *Panulirus interruptus*. II. Chemical and antigenic relation to blood proteins. *Biol. Bull. (Woods Hole, Mass.)* **89**, 193–200.
- Tyson, C., and Jenkin, C. (1973). The importance of opsonic factors in the removal of bacteria from the circulation of the crayfish, *Parachaeraps bicarinatus*. *Aust. J. Exp. Biol. Med. Sci.* **51**, 609–618.
- Tyson, C. J., and Jenkin, C. R. (1974). The cytotoxic effect of haemocytes from the crayfish (*Parachaeraps bicarinatus*) on tumour cells of vertebrates. *Aust. J. Exp. Biol. Med. Sci.* **52**, 915–926.

- Unestam, T., and Nylund, J.-E. (1972). Blood reactions *in vitro* in crayfish against a fungal parasite, *Aphanomyces astaci*. *J. Invertebr. Pathol.* **19**, 94–106.
- Valiulis, G. A. (1973). "Comparison of the Resistance to *Labyrinthomyxa marina* with Resistance to *Minchinia nelsoni* in *Crassostrea virginica*," Ph.D. dissertation. Rutgers University, New Brunswick, New Jersey.
- Valiulis, G. A., and Haskin, H. H. (1973). Resistance of *Crassostrea virginica* to *Minchinia nelsoni* and *Labyrinthomyxa marina*. *Proc. Natl. Shellfish. Assoc.* **63**, 6.
- van der Knaap, W. P. W., Boerrigter-Barendsen, L. H., van den Hoeven, D. S. P., and Sminia, T. (1981a). Immunocytochemical demonstration of a humoral defence factor in blood cells (amoebocytes) of the pond snail *Lymnaea stagnalis*. *Cell Tissue Res.* **219**, 291–296.
- van der Knaap, W. P. W., Doderer, A., Sminia, T., and Boerrigter-Barendsen, L. H. (1981b). Some properties of an agglutinin in the haemolymph of the pond snail *Lymnaea stagnalis*. *Biol. Bull. (Woods Hole, Mass.)* **162**, 404–412.
- van der Knaap, W. P. W., Doderer, A., Boerrigter-Barendsen, L. H., and Sminia, T. (1982). Some properties of an agglutinin in the haemolymph of the pond snail *Lymnaea stagnalis*. *Biol. Bull. (Woods Hole, Mass.)* **162**, 404–412.
- Warr, G. W. (1981). Immunity in invertebrates. *J. Invertebr. Pathol.* **38**, 311–314.
- Weinheimer, P. F., Acton, R. T., and Evans, E. E. (1969a). Attempt to induce a bactericidal response in the oyster. *J. Bacteriol.* **97**, 462–463.
- Weinheimer, P. F., Acton, R. T., Sawyer, S., and Evans, E. E. (1969b). Specificity of the induced bactericidin of the West Indian spiny lobster, *Panulirus argus*. *J. Bacteriol.* **98**, 947–948.
- Weinheimer, P. F., Evans, E. E., Acton, R. T. and Painter, B. (1969c). Induced response of the lobster, *Panulirus argus*. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **28**, 752.
- Weinheimer, P. F., Evans, E. E., Stroud, R. M., Acton, R. T., and Painter, B. (1969d). Comparative immunology: Natural hemolytic system of the spiny lobster, *Panulirus argus*. *Proc. Soc. Exp. Biol. Med.* **130**, 322.
- Wittke, M., and Renwantz, L. (1984). Quantification of cytotoxic hemocytes of *Mytilus edulis* using a cytotoxicity assay in agar. *J. Invertebr. Pathol.* **43**, 248–253.
- Wood, P. C. (1965a). A preliminary note on gaffkaemia investigations in England. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **156**, 30–34.
- Wood, P. C. (1965b). Gaffkaemia, the blood disease of lobsters. *J. Gen. Microbiol.* **41**, 28.
- Wood, W. B., Jr. (1953). Studies on the cellular immunology of acute bacterial infections. *Harvey Lect.* **47**, 72–98.
- Wood, W. B., Jr., Smith, M. R., and Watson, B. (1946). Studies on the mechanism of recovery in pneumococcal pneumonia. IV. The mechanism of phagocytosis in the absence of antibody. *J. Exp. Med.* **84**, 387–402.
- Wright, A. E., and Douglas, S. R. (1903). An experimental investigation of the role of the blood fluids in connection with phagocytosis. *Proc. R. Soc. London, Ser. B* **72**, 357–370.
- Yoshino, T. P., and Cheng, T. C. (1976). Fine structural localization of acid phosphatase in granulocytes of the pelecypod *Mercenaria mercenaria*. *Trans. Am. Microsc. Soc.* **95**, 215–220.
- Yoshino, T. P., and Tuan, T. L. (1985). Soluble mediators of cytolytic activity in hemocytes of the Asian clam, *Corbicula fluminea*. *Dev. Comp. Immunol.* **9**, 515–522.

Part V

Diseases of Captive Shellfish

Diseases can appear in shellfish populations held or grown under the following artificial conditions:

1. Short-term holding of market-size crustaceans such as lobsters and crabs in pounds, tanks, and live cars; and similar holding of market-size clams and oysters in tanks, trays, and inshore beds
2. Cultivation of shellfish such as oysters or mussels in natural waters, which involves catching seed in natural waters; spreading seed on growing beds, or transferring it to growing racks or rafts; and possibly transfer of partially grown stocks to other growing beds; or, in Crustacea, the cultivation of shrimp by trapping larvae or juveniles in tidal ponds and utilizing natural productivity or adding nutrients to rear market-size animals
3. Production of molluscan shellfish larvae and spat in hatcheries, or the production of crustacean larvae and juveniles in hatcheries, in treated flow-through or recirculated seawater, followed in some cases by growth to market size under artificial conditions (raceways, ponds, net enclosures)

Chapter 11 emphasizes diseases encountered in holding and maintaining captive populations acquired at or near market size from wild stocks, leaving the much larger and more exciting field of disease in cultured shellfish to be considered in Chapter 12.

Disease in Impounded Populations

I. INTRODUCTION

Molluscan and crustacean shellfish may be impounded for a variety of reasons:

- Molluscs (clams and oysters) may be held in large seawater tanks of depuration facilities, which exist in a number of industrialized countries. The concept underlying their operation is short-term (24–72 hours) holding of clams or oysters in ozone- or ultraviolet-treated seawater, to reduce coliform bacterial contamination (and hopefully other types of microbial contamination). Some depuration plants—especially those in France—have large capacities and are highly automated.
- Molluscs (hard clams in particular) may be held in trays or shallow near-shore plots for periods of days or weeks to await improvement in market conditions or to await sorting by size.
- Premolt crabs may be held in seawater tanks until they have molted, at which time they are removed and sold as soft-shell crabs. One of the most extensive holding operations for crustaceans is that associated with “shedding” of blue crabs, *Callinectes sapidus*, for the soft-shell crab industry of the east coast of the United States. Premolt crabs are captured and held in seawater tanks until they molt; the soft crabs are then processed and sold, or simply refrigerated and sold. Crabs are under stress, of course, from initial capture, crowding in tanks, and physiological changes during molting. Mortalities are often high, from non-optimum holding conditions or from disease—either preexisting or acquired in the tanks.
- Lobsters may be held in floating live cars or enclosed tidal pounds for weeks or months to await improvement in market conditions. During this period the animals may or may not be

fed, and some attention is usually given to oxygen levels. Cannibalism is a problem, as are other forms of mortality, including disease.

Whereas these are some of the most common examples of impoundment of shellfish, other species may be held for varying periods for a variety of reasons, usually associated with marketing. Mortalities are, of course, always a concern, since they represent lost revenue; disease-caused mortalities may sometimes assume significant proportions, especially if the animals are already weakened or if the holding period is at all extensive.

II. TYPES OF DISEASE CONDITIONS SEEN IN IMPOUNDED POPULATIONS

From experiences with short-term holding of molluscan and crustacean shellfish for eventual sale and consumption, three principal categories of disease problems have become apparent:

1. Animals captured from wild stocks may already be infected by a disease agent; the stresses of capture and confinement can exacerbate the condition, often causing mortality.
2. Animals from wild stocks may be injured during capture or transport, with wounds providing portals of entry for opportunistic invaders.
3. Captive but intact animals may become infected with rapidly multiplying facultative microbial pathogens, such as vibrios, which may be lethal in a matter of days, especially if environmental conditions in the holding facility are poor or marginal.

These categories are of course not mutually exclusive, but can be useful in describing disease conditions seen in holding facilities.

The *presence of preexisting infections* (category 1)—latent, chronic, or acute—can do much to ensure the early demise of captive animals. The combination of a virulent pathogen already established in the host and stresses of confinement provides the proper scenario for the development of acute infections and death, often in a matter of hours or days.

One of the best examples of this category of disease problem can be seen in the “gray crab” disease of premolt blue crabs being held in shedding tanks. The protozoan pathogen, *Paramoeba perniciosa*, may occur in epizootic levels in wild populations. Infected crabs, weakened and debilitated even before capture, succumb quickly in the tanks as the disease agent proliferates rapidly in the hemolymph and the connective

tissues. Mortalities in holding facilities during epizootic periods have been reported to be as high as 30% (Newman and Ward, 1973). The highest prevalences in wild populations (up to 57% in one survey) occurred during the early summer peak of molting, although subacute infections were found year-round (Johnson, 1977; Couch, 1983).

Category 2—diseases which are consequences of injuries sustained during capture or transport, or from fighting among cohorts in crowded holding tanks—includes several of the well-known diseases of lobsters. Populations of American lobsters, *Homarus americanus*, held in live cars or pounds suffer major losses from two bacterial diseases: red tail disease or gaffkemia, caused by *Aerococcus viridans*, and shell disease, caused by chitin-destroying, gram-negative bacilli (considered in greater detail in Chapter 3). Both diseases can be transmitted under crowded conditions that prevail in pounds, and both diseases seem to require some break in the exoskeleton for entry of the pathogens. Gaffkemia was first noted on the North American east coast in 1946. Disease signs include variable pink coloration of the ventral abdomen, pink hemolymph, prolonged clotting time, and drastic reduction in blood phagocytes. Diseased lobsters become progressively weaker, and mortalities may reach 50% after short periods of storage. Mortalities increase sharply when water temperature exceeds 15°C.

Two epizootics of gaffkemia have occurred on the Maine coast, one in 1946–1947 and another in 1959–1960 (Goggins and Hurst, 1960). Losses reached as high as 58% of the impounded populations. The pathogen, *A. viridans*, was able to live and multiply outside the lobster, in the slime on lobster cars, crates, tanks, and live wells. The organism was also isolated from the mud of tidal pounds and from seawater several miles from infected pounds. The disease was transmitted experimentally by allowing presumably healthy lobsters to feed on infected individuals or by holding healthy lobsters in seawater containing the pathogen. Deaths followed in 14–21 days. Treatment of tidal pounds with calcium hypochlorite reduced populations of the pathogen in bottom mud, and reduced subsequent losses of impounded lobsters.

Aerococcus viridans has also been isolated from European lobsters, *Homarus gammarus*, by Wood (1965). He observed mortalities in storage tanks in southern England in 1962 and recovered organisms with cultural and biochemical characteristics similar to Canadian and U.S. isolates. *Aerococcus viridans* has also been isolated from impounded *H. gammarus* and imported *H. americanus* in Norway (Håstein *et al.*, 1977; Wiik *et al.*, 1987) and from impounded lobsters in France (Audouin and Leglise, 1971).

The second bacterial disease of lobsters, known as shell disease, is

caused by chitin-destroying, gram-negative bacilli. Hess (1937) isolated chitin-degrading bacteria from live American lobsters impounded at Yarmouth, Nova Scotia. The disease was characterized by a pitting and sculpturing of the exoskeleton. Initial lesions occurred on the walking legs and were distinguished by white outer margins, from which the bacteria were most readily isolated. Although disease signs were first seen in impounded lobsters, similar conditions were later observed in newly caught lobsters from several widely separated Canadian fishing grounds. Hess found the disease to be relatively rare in natural populations but noted severe shell erosion and weakening of lobsters stored in pounds over the winter.

Significant mortalities of captive lobsters may accompany the shell disease. Taylor (1948) stated that 71% of infected captive lobsters died from the disease, but observed no correlation between mortality and the intensity of external shell erosion. Acquisition of the disease by healthy lobsters placed in seawater tanks with infected individuals indicated direct transmission. The disease developed slowly, requiring at least 3 months before the advanced stages were reached. Progress of chitin destruction was directly temperature dependent and new shell laid down after molting was not affected, except by reinfection. Gill erosion was considered a principal reason for mortalities.

Subsequent studies have implicated chitinoclastic members of three bacterial genera—*Vibrio*, *Pseudomonas*, and *Aeromonas*—in shell disease of lobsters and have reaffirmed the need for a damaged cuticle to achieve experimental infections (Malloy, 1978; Roald *et al.*, 1981).

It seems quite probable that both gaffkemia and shell disease may already be present in some individual lobsters prior to capture, but, at least for gaffkemia, in which the disease course is rapid and injuries are common in captivity, it is quite feasible for lethal infections to be acquired after capture.

Category 3—*diseases caused by facultative microbial pathogens*—can be distinguished from category 2 only in that the animal may be stressed, but may not necessarily be injured (in the sense that integrity of the integument is destroyed). Members of the bacterial genus *Vibrio* (and related genera *Pseudomonas* and *Aeromonas*) are the best-known representatives of this category, exerting their influence wherever animals are held under abnormal conditions of nutrition, temperature, salinity, water quality, and population density.

Vibrio infections have been reported in lobsters being held for extended periods. *Vibrio parahaemolyticus* and the closely related *Vibrio alginolyticus* were isolated from moribund aquarium-held lobsters in Canada (Brinkley *et al.*, 1976). The authors speculated that stress due to

acclimation to high temperatures (22°C) could have predisposed the animals to infections and contributed to deaths. The facultative pathogens were thought to have been introduced into the tanks in association with the lobsters, which had been acquired from commercial sources.

Vibrio infections are particular problems in blue crab shedding operations. In one study 91% of the crabs had *Vibrios* (*V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae*) present in the hemolymph (Sizemore, 1986). In another study *Vibrio* spp. were found to be common inhabitants of blue crab hemolymph, with invasion considered most likely during molting or as a result of injury (Sizemore and Davis, 1985). Some reduction in infections with *V. parahaemolyticus* and *V. vulnificus* was effected by holding crabs in low salinities (Overstreet, 1986). An extensive seasonal study of *Vibrio* infections in blue crabs disclosed a marked peak in summer, with abundant disease and mortalities during the first 10 days after capture (Johnson, 1976). The author concluded that the bacterial infections were acquired principally during capture and transport, from potentially pathogenic agents in the water or on the exoskeleton.

Other types of facultative microorganisms can cause distress and even mortalities in blue crabs held for shedding. The practice of holding crabs aids the buildup of commensal ciliates on the gills and contributes to mortalities. Molting blue crabs from Chesapeake Bay suffered serious mortalities in the summers of 1965 and 1966. Their gills had a massive infestation of peritrichous ciliates of the genera *Lagenophrys* and *Epistylis* (Couch, 1966, 1967). Mortalities were most severe among crabs in holding tanks just before or after molting, but crabs from natural waters were also heavily infested, and fisherman reported mortalities. Infestations of gills frequently seemed heavy enough to interfere with respiration. (It is quite possible, of course, that some mortalities in shedding tanks were caused by the protozoan *Paramoeba perniciosus*, which was also epizootic at that time.)

Other microbial epibionts have been reported from captive crustaceans. Most conspicuous are filamentous forms such as the bacterium *Leucothrix mucor* and algae such as *Oscillatoria* and *Anabaena* (Fisher *et al.*, 1978).

It should be very obvious by now that the examples given in this chapter are confined to crustaceans, mainly because this is where the problems exist. Molluscan shellfish are impounded or stored for short periods to take advantage of market conditions or for depuration, but diseases have not often been recognized as causes of mortalities during storage, even though they may be important in molluscan culture, especially in larvae and juveniles.

There is some limited information, though. One intriguing example

is West River disease, seen in Canadian oysters (*Crassostrea virginica*) kept in cold-air storage. Many oysters (up to 25%) died during 1948–1954 soon after placement in storage. Meats had a characteristic green–black color and a peculiar offensive odor. The condition was seen only in oysters from one location on Prince Edward Island; moribund oysters before harvest exhibited a progressive blackening of the gills, accompanied by erosion of the digestive epithelium. The condition, thought to be an infectious disease but possibly of other etiology, disappeared in 1954 (Logie, 1956).

Another example of disease in impounded molluscan populations was reported from Japan (Hatai, 1982). Abalone, *Haliotis sieboldii*, being held in a storage facility, experienced an epizootic caused by the fungus *Haliphthoros milfordensis*, known principally as a pathogen of larval crustaceans.

It can be concluded from these selected examples that there is little that is unique about the diseases of impounded shellfish. They are usually reflections of infectious diseases in wild populations, enhanced by close confinement of hosts under highly abnormal environmental conditions and augmented by activities of facultative pathogenic microorganisms.

III. DISEASE CONTROL METHODS IN IMPOUNDED POPULATIONS

Disease-caused mortalities are clearly undesirable accompaniments of short-term storage of market-sized shellfish. Because of the substantial existing investment in such products, any reasonable steps to reduce losses due to disease in impoundment facilities make good economic sense. Some of the more important measures to be taken to minimize losses are:

- The period of impoundment should be kept to a minimum, consistent with favorable market position.
- Sick animals should not be impounded, especially during epizootic periods, when disease prevalences in wild stocks may be high.
- If animals are to be held for extended periods, they should be fed.
- Population densities in holding facilities should be limited and cover should be provided for aggressive and/or cannibalistic species, such as lobsters.
- Water quality in holding facilities should be maintained at a high level (critical elements, which should be monitored closely, are

oxygen, salinity, temperature, ammonia, and the presence of industrial pollutant chemicals).

- Water circulation should be adequate to prevent the existence of pockets or strata of unfavorable environmental conditions in holding tanks.
- If abnormal behavioral or other disease signs appear or if unusual levels of mortality are noted, the impounded population should be sold or processed quickly, to minimize losses.
- Bacterial levels in holding tanks should be kept low, by filtration, ozonization, or ultraviolet sterilization.
- Corpses, which may be sources of pathogens, should be removed from holding tanks promptly and routinely.
- Sources of advice on disease problems—microbiologists, pathologists—are scarce but precious commodities in crisis situations. Cultivate and treasure them.

Other admonitions could be proposed, but most of these are matters of common sense, combined with good facility engineering and sanitation practices. Disease problems in impounded populations results either from preexisting conditions in the wild stock or from stresses imposed by the holding environment—or from a combination of the two.

IV. CONCLUSIONS

Disease problems in impounded molluscan and crustacean shellfish populations are derived from preexisting infections in wild-caught individuals and from rapid proliferation of facultative microbial pathogens, with or without injuries as entry points. Preexisting infections, often with virulent pathogens, may be present at enzootic or epizootic levels in the source population. Such pathogens may act rapidly in the abnormal holding environment to kill already infected individuals and to infect others. Facultative microbial pathogens may be present in or on the animals at the time of capture, or they may be present in the seawater brought into the holding facility. Their pathogenesis is facilitated by injuries or by the debilitating effects of stress on the host animals.

REFERENCES

- Audouin, J., and Leglise, M. (1971). Note préliminaire sur la présence de *Gaffkya homari* dans le sang des homards stockés dans les viviers de la région de Roscoff. *Int. Counc. Explor. Sea, Doc. C.M.1971/K:26*, 6 pp.
- Brinkley, A. W., Rommel, F. A., and Huber, T. W. (1976). The isolation of *Vibrio para-*

- haemolyticus* and related vibrios from moribund aquarium lobsters. *Can. J. Microbiol.* **22**, 315–317.
- Couch, J. A. (1966). Two peritrichous ciliates from the gills of the blue crab. *Chesapeake Sci.* **7**, 171–176.
- Couch, J. A. (1967). A new species of *Lagenophrys* (Ciliata: Peritrichida: Lagenophryidae) from a marine crab, *Callinectes sapidus*. *Trans. Am. Microsc. Soc.* **86**, 204–211.
- Couch, J. A. (1983). Diseases caused by Protozoa. In "The Biology of Crustacea: Vol. 6. Pathobiology" (A. Provenzano, ed.), pp. 79–111. Academic Press, New York.
- Fisher, W. S., Nilson, E. H., Steenbergen, J. F., and Lightner, D. V. (1978). Microbial diseases of cultured lobsters: A review. *Aquaculture* **14**, 115–140.
- Goggins, P. L., and Hurst, J. W., Jr. (1960). Progress report on lobster gaffkyremia (red tail). *Bull. Maine Dep. Sea Shore Fish.* 9 pp. (unpublished mimeo).
- Håstein, T., Roald, S. O., Kjos-Hansen, B., and Staveland, K. (1977). Occurrence of gaffkemia in lobsters in Norway. *Acta Vet. Scand.* **18**, 138–139.
- Hatai, K. (1982). On the fungus *Haliphthoros milfordensis* isolated from temporarily held abalone (*Haliotis sieboldii*). *Gyobyo Kenkyu* **17**, 199–204 (in Japanese; English abstract).
- Hess, E. (1937). A shell disease in lobsters (*Homarus americanus*) caused by chitinovorous bacteria. *J. Biol. Board Can.* **3**, 358–362.
- Johnson, P. T. (1976). Bacterial infection in the blue crab, *Callinectes sapidus*: Course of infection and histopathology. *J. Invertebr. Pathol.* **28**, 25–36.
- Johnson, P. T. (1977). Paramoebiasis in the blue crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* **29**, 308–320.
- Logie, R. R. (1956). Oyster mortalities, old and new, in the Maritimes. *Fish. Res. Board Can., Prog. Rep. Atl. Coast Stn.* **65**, 3–11.
- Malloy, S. C. (1978). Bacteria induced shell disease of lobsters (*Homarus americanus*). *J. Wildl. Dis.* **14**, 2–10.
- Newman, M. W., and Ward, G. E., Jr. (1973). An epizootic of blue crabs, *Callinectes sapidus*, caused by *Paramoeba pernicio*s. *J. Invertebr. Pathol.* **22**, 329–334.
- Overstreet, R. M. (1986). Solving parasite-related problems in cultured Crustacea. In "Parasitology—Quo Vadit?" (M. J. Howell, ed.), Proc. 6th Int. Congr. Parasitol. pp. 309–318. Aust. Acad. Sci., Canberra, Australia.
- Roald, S. O., Aursjø, J., and Håstein, T. (1981). Occurrence of shell disease in lobsters, *Homarus gammarus* (L.) in the southern part of Oslofjord, Norway. *Fiskeri dir. Skr., Ser. Havunders.* **17**, 153–160.
- Sizemore, R. K. (1986). Involvement of *Vibrio* spp. in soft crab mortality. In "Proceedings of the National Symposium of the Soft-Shell Blue Crab Fishery" (H. M. Perry and R. F. Malone, eds.), pp. 21–22. Louisiana State University, Baton Rouge, Louisiana.
- Sizemore, R. K., and Davis, J. W. (1985). Source of *Vibrio* spp. found in the hemolymph of the blue crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* **46**, 109–110.
- Taylor, C. C. (1948). Shell disease as a mortality factor in the lobster (*Homarus americanus*). *Maine Dep. Sea Shore Fish. Circ.* **4**, 8 pp.
- Wiik, R., Egidius, E., and Goksøyr, J. (1987). Screening of Norwegian lobsters *Homarus gammarus* for the lobster pathogen *Aerococcus viridans*. *Dis. Aquat. Org.* **3**, 97–100.
- Wood, P. C. (1965). A preliminary note on gaffkemia investigations in England. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **156**, 303–304.

12

Disease in Shellfish Culture

I. INTRODUCTION

Some of the great advances in the history of agriculture have been associated with improved disease and pest control, combined with genetic improvement of strains and stocks of plants and animals. Production from animal husbandry has been enhanced by measures to control diseases and parasites, and the production of staple crops has been maintained and in some instances expanded enormously by the control of diseases and pests, but the struggle against disease in agriculture has been continuous: new problems appear as old ones are attacked and solved (or at least controlled).

Aquaculture, as a counterpart of agriculture, has had and will have a counterpart history in terms of disease effects and disease control. The concepts and operating principles are the same: create an artificial (and at times stressful) environment, select for high yield, and crowd the animals (even though high densities increase the likelihood of disease transmission and mortalities). These activities lead to the necessity to select for disease resistance, immunize the animals, or apply chemical treatments (or all of the above)—methods to block disease-caused natural population density regulation and to reduce mortalities to a point at which profits can be made.

What is the record of success and failure in understanding and controlling diseases in shellfish aquaculture? The early record was not outstanding; the current record is better, but not greatly so. In open extensive culture of oysters, for example, great mortalities ravaged and still ravage cultivated populations (Sindermann, 1979). Each decade has seen increased scientific attention to and augmentation of information about pathogens and how to limit their effects in aquaculture. There has been a proportionate increase in successes over failures.

Many hazards confront those resourceful enough to attempt to pro-

duce significant quantities of food from marine aquaculture. Constraints and problems can be categorized as economic, legal, or technical. Among those of a technical nature, none has proven to be more severe, or resistant to resolution, than disease. Entire aquaculture operations have persisted or disappeared based on success or failure in the reduction of disease-caused mortality to acceptable levels. Methods for disease diagnosis and control for marine aquaculture have improved during the past two decades (Sindermann, 1970a; Vivares *et al.*, 1986; Sindermann and Lightner, 1988), although much remains to be accomplished.

Marine aquaculture, as it is practiced today, can be artificially divided into *extensive aquaculture*, utilizing natural bodies of water, with only modest technological sophistication, with little environmental control, and with low grow-out densities, and *intensive aquaculture*, in artificial bodies of water (raceways, tanks, constructed ponds), with higher levels of environmental control, higher technological applications, and high grow-out densities. Extensive culture would include oyster culture, as conducted in many countries, and ocean ranching of shrimp. Intensive culture would include shrimp culture in large circular tanks, as used in Japan.

The technological base of aquaculture—especially that concerned with hatching and early rearing—is expanding each year, and various shellfish can now be grown to market size under artificial conditions. Disease research has contributed to the expanding technological base, since, regardless of the degree of sophistication in other methodology, adequate disease control must be available if catastrophic losses are to be avoided. Hatcheries have become integral parts of shellfish culture systems—especially for shrimp, oysters, and clams—even though the natural production of seed is still important.

Among the bivalve molluscs, a pressing need for disease control exists in all hatchery operations. Early in the history of the development of molluscan hatcheries, Tubiash *et al.* (1965) described mass mortalities of bivalve mollusc larvae in hatchery tanks due to gram-negative, motile bacilli believed to be *Vibrio* sp. or *Aeromonas* sp. (Fig. 43) and found that all of the pathogenic serotypes were sensitive to certain antibiotics. Combistrep and chloramphenicol were effective as therapeutic agents. Since the early studies by Tubiash and associates, other *Vibrio* strains have been isolated from molluscan hatchery disease outbreaks (Helm and Smith, 1971; Brown, 1981), and several shellfish-pathogenic strains have been aggregated into a new species *Vibrio tubiashii* (Hada *et al.*, 1984). Other epizootics in hatchery-reared oyster and clam larvae were found by Davis *et al.* (1954) to be caused by a fungus, later described by

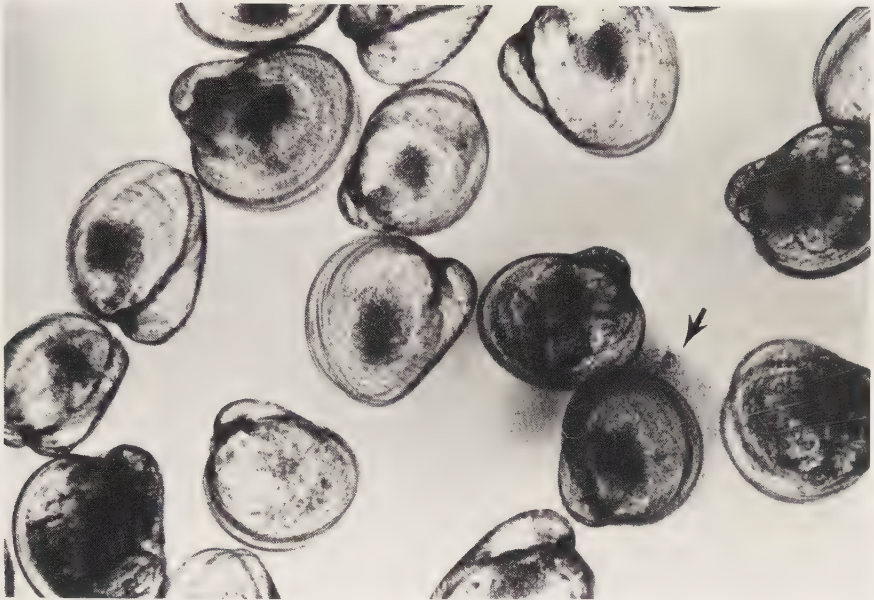


Figure 43. The effects of bacterial disease in hatchery-reared bivalve larvae. Note dead individuals and bacterial "swarming" in the center of the photograph. From Tubiash *et al.* (1965).

Vishniac (1955) as *Sirolpidium zoophthorum*. Juvenile as well as larval bivalves were killed, and epizootics in particular cultures destroyed most of the populations in 2–4 days. Some control of epizootics, or reduction of their effects, can be achieved by such measures as filtration and ultraviolet treatment of seawater and the use of bacteria-free algal cultures or sterilized artificial diets.

Hatcheries for the production of lobster larvae have been in existence in the United States and elsewhere for over half a century. Techniques were developed to hold "berried" (egg-bearing) females until larvae hatched and to carry the larvae through several molts. The larvae were then returned to the sea, but produced no demonstrable effect on the abundance of natural populations. Because of the high individual value of species such as lobsters, techniques have been developed in recent years that enable rearing of lobsters under aquaculture conditions, although the cost of production still exceeds any market value. In the now discarded practice of short-term hatching and the early release of lobster larvae—and especially in presently available systems of rearing to market size—diseases and parasites have been problems. Egg-bearing female lobsters held in hatching troughs may die from the ef-

fects of *Aerococcus* infections before their eggs are hatched. Similarly, female lobsters, as well as their eggs and larvae, may be infested with the annelid *Histriobdella*. Lobster eggs may also be destroyed by the suctorian *Ephelota*. Pilot-scale rearing of lobsters has encountered other diseases—especially those of bacterial, fungal, and protozoal stiology—that can affect various early life stages.

Shrimp culture, using hatcheries and various kinds of grow-out ponds, tanks, raceways, and net enclosures, has also been affected by disease problems. The significance of disease in early attempts to rear prawns, *Palaemon serratus*, was reported by Anderson and Conroy (1968). Prawns were severely affected by a progressive systemic fungus disease which was first seen in egg-bearing females which had been captured at sea. Infection quickly spread to most groups of prawns being cultured. The etiological agent was tentatively assigned to the phycomycete genus *Pythium*, and the authors noted the similarity between this disease and a mycosis caused by *Aphanomyces astaci* in European freshwater crayfish. Another important pathological condition seen in the prawns was brown spot disease, characterized by erosion of the exoskeleton and inflammation of the underlying tissues. The disease was widespread among the cultured prawns. Anderson and Conroy alluded to other published examples of crustacean diseases caused by chitinovorous bacteria, but did not identify the organism responsible for brown spot disease. The concluding statement by Anderson and Conroy is of interest: "In any large-scale crustacean hatchery, diseases will undoubtedly occur and practical remedies will have to be sought. Mycoses would appear to be particularly hazardous and as far as we are aware no effective therapy is currently available." Since that early work, disease has continued to be a problem in crustacean culture. A recent book on diseases in marine aquaculture lists 28 diseases or disease conditions now known in penaeid shrimp culture (Sindermann and Lightner, 1988). In accord with the prediction by Anderson and Conroy, (1968), fungal infections have proved to be important, as have viral infections.

This chapter has four principal sections: (1) an attempt to examine the reality of disease problems in selected examples of shellfish culture—oysters, shrimp, and lobsters—emphasizing quantitative information when available; (2) an assessment of disease effects on cultured populations of shellfish; (3) a detailed consideration of principles and mechanisms of disease control in shellfish culture; and (4) a summarization of some current concepts about the role of disease in cultured shellfish species. Emphasis is clearly on the more pragmatic aspects of disease effects and disease control in aquaculture populations.

II. "CASE HISTORIES" OF THE EFFECTS OF DISEASE ON SHELLFISH CULTURE

To illustrate the nature and the extent of disease impacts on aquaculture, examples have been selected from among those species which have been cultured successfully, or for which reasonably adequate information exists. Of the available choices, oysters, shrimp, and lobsters have been selected, as illustrative of the progress made during the past several decades in understanding and controlling diseases of cultivated shellfish.

A. Oysters

Oyster populations throughout the world have been characterized by mass mortalities, some of which have been attributed to epizootics of specific pathogens (Sindermann, 1970b, 1979). Although roughly 70% of the earlier mass mortalities could not be specifically associated with a cause, disease or otherwise, in recent decades there have been several episodes in which deaths were clearly linked to epizootics of viral and protistan pathogens.

Beginning in the late 1940s, mortalities due to epizootic diseases occurred in oyster (*Crassostrea virginica*) populations on the Atlantic and Gulf of Mexico coasts of North America. Initial studies in the Gulf (Mackin *et al.*, 1950; Ray, 1954; Mackin, 1962) identified a fungus pathogen (*Dermocystidium marinum*—now the protozoan *Perkinsus marinus*) as the causative agent. Direct transmission was demonstrated, and a management scheme based on biological characteristics of the pathogen reduced the effects of the disease on oyster production. (The pathogen, is, however, still a significant mortality factor in many growing areas.)

Then, in the late 1950s new and drastic mortalities began in Delaware and Chesapeake Bays, the principal oyster-producing areas of the Middle Atlantic coast. Intensive studies by federal, state, and university investigators disclosed the presence of two protozoan (haplosporidan) pathogens, *Haplosporidium nelsoni* and *Haplosporidium costale* (the former being more important). Mortalities in the early years of the epizootics reached 90% on some beds and have continued at a variable level to the present time. Oyster production declined sharply, especially in Delaware Bay, and has remained depressed in the presence of the pathogen *H. nelsoni* (Haskin and Ford, 1983). Much has been learned about the pathogen, but its life cycle and method of transmission are still problematic, despite extensive literature and 25 years of study (Wood and

Andrews, 1962; Couch *et al.*, 1966; Haskin *et al.*, 1966; Farley, 1967; Andrews and Wood, 1967; Andrews and Frierman, 1974; Andrews, 1966, 1968, 1979; Haskin and Ford, 1979, 1982; Ford and Haskin, 1982; and others). The pathogen is intolerant of extended salinities below 15 ppt, which has enabled the development of some management methods to reduce mortalities. Also, some evidence for cyclic activity has been found.

During the late 1960s and the 1970s, epizootics of similar proportions occurred on the northern coast of France, involving two intensively cultivated species of oysters, the Portuguese oyster (*Crassostrea angulata*) and the European flat oyster (*Ostrea edulis*). Mortalities, beginning in the late 1960s, were caused by successive epizootics of quite distinct pathogens. Abnormal mortalities of Portuguese oysters, *C. angulata*, were first noted on the Atlantic coast of France in the summer of 1966 (Marteil, 1969; Comps *et al.*, 1976). Diseased oysters had extensive lesions of the gills and palps. In the growing areas most affected, as many as 80% of the oysters were infected, and the overall mortality associated with this gill disease was estimated at 40%. Early in the study of the mortalities, the etiological agent was thought to be a new protistan pathogen, *Thanatostrea polymorpha*, but later studies disclosed viral (iridovirus) infections (Comps and Duthoit, 1976). Gill disease was also observed in Spain, Portugal, and the United Kingdom.

Then, beginning in 1970 new mortalities occurred in *C. angulata* populations, apparently caused by another iridovirus (Comps *et al.*, 1976). Mortalities continued through 1973 and then subsided. There remains some question as to whether two separate epizootics (1966–1969 and 1970–1973) occurred (and some doubt about the etiology of the 1966–1969 outbreak), but the reality was the almost complete destruction of *C. angulata* populations on the French coast due to epizootic disease. The diminished *C. angulata* populations were replaced by massive importation of Pacific oysters, *Crassostrea gigas*, from Japan and from British Columbia (Canada).

During the same period and in the same area, populations of European flat oysters, *O. edulis*, were also reduced by mortalities resulting from epizootic disease. Beginning in 1968, in estuaries on the French Channel coast, a protistan parasite, *Marteilia refringens*, began affecting *O. edulis* populations (Comps, 1970; Grizel *et al.*, 1974). The pathogen eventually invaded all oyster-growing areas on the Channel and Atlantic coasts of France. The focus of infection was in the digestive gland tubules. The epizootic, with accompanying mortalities, reached a peak in the early 1970s and declined from 1977 on. As the epizootic due to *M. refringens* in *O. edulis* waned in the late 1970s and populations began to

be rebuilt, another protistan parasite, *Bonamia ostreae*, was identified as the cause of new mortalities (Comps *et al.*, 1980; Pichot *et al.*, 1980, 1981). Beginning in the summer of 1979 on the Brittany coast, the disease spread to Normandy and Arcachon and continues at epizootic levels at the present time (1989). *Bonamia* disease has been reported in The Netherlands, Denmark, and Spain as well.

The occurrence, within a single decade, of at least three and possibly four epizootics, each with accompanying extensive mortalities, is unique in the long history of oyster cultivation. Also unique is the scale of importation of a replacement species (the Pacific oyster) during the early 1970s especially. The role that this massive introduction of a nonindigenous species may have played in any of the disease outbreaks is unknown, despite extensive scientific study of the epizootics and the pathogens. Pacific oysters are reproducing in some culture areas, and have largely replaced the Portuguese oysters on most grow-out beds.

Partly as a result of scientific study, and partly through trial and error, control measures to alleviate disease impacts were developed and instituted for at least one of the pathogens: *M. refringens*. Infection was found to occur in July and August, so the absence of planting during that period reduced the risk of infection of seed oysters. This kind of disease control—making use of information about the physiological and environmental requirements of the pathogen—was applied successfully in combating protistan parasites of oysters on the North American East Coast during the 1960s. *Haplosporidium nelsoni*, a haplosporidan protozoan recognized as the causative organism of Delaware Bay disease of American oysters, *C. virginica*, was found to be intolerant of low salinity and infective only during the warmer months of the year. A pattern of seed planting combined with early harvest was developed, which reduced disease impact in epizootic areas in the Middle Atlantic states and encouraged production in low-salinity zones of upper estuaries. Another protistan pathogen, *Perkinsus marinus*, causes significant oyster population reductions in the Gulf of Mexico during the warmer months of the year. Direct transfer of the disease organism in dense populations of oysters was reduced by planting thinly, and seed planting in high-salinity waters avoided the summer period. These techniques have been used for over two decades as methods of stock management.

B. Shrimp

Because of the great market potential of shrimp, particularly larger members of the genus *Penaeus*, attempts have been made to augment natural production by pond, lagoon, and tank culture, building on culture

methods which have been used in Asia for centuries. Shrimp culture is expanding in many parts of the world, and pilot-scale activities are developing in still other locations. With this increased effort has come recognition of a number of diseases, some of which have caused mortalities in cultivated populations.

As is true in most of marine aquaculture, microbial diseases have been described, including those of viral, bacterial, and fungal etiology. A baculovirus (*Baculovirus penaei*) was described (Couch, 1974) as a pathogen of penaeid shrimp (*Penaeus duorarum* and *Penaeus aztecus*) from the Gulf of Mexico, and a counterpart viral pathogen was recently described in cultured Kuruma prawn (*Penaeus japonicus*) from Japan (Sano *et al.*, 1981). There is some evidence that the Gulf of Mexico virus may cause epizootic mortalities in wild shrimp populations (Couch *et al.*, 1975), and the Japanese report described mortalities of larvae and postlarvae which have occurred since 1971, severe enough so that "it was impossible to provide a stable supply of shrimp seeds to the culture farms." "Oral inoculation" was successful in transmitting the Japanese virus; no polyhedral inclusion bodies were noted, unlike those characteristic of the American shrimp baculovirus.

Vibrios are important in the ecology and survival of cultivated shrimp populations. *Vibrio parahaemolyticus*, which has been associated with human gastroenteritis in several countries, was found by Vanderzant *et al.* (1970) to be lethal to experimental populations of brown shrimp, *P. aztecus*. Subsequent studies have shown that other vibrios (e.g., *Vibrio anguillarum* and *Vibrio alginolyticus*) can cause epizootics and mortalities of brown, pink, (*P. duorarum*) and white shrimp (*P. setiferus*) (Lewis, 1973; Lightner and Lewis, 1975). Mortalities attributable to *V. alginolyticus* infection were reported in 1972 and in 1973 in a commercial hatchery in the United States (Lightner, 1975). Both white and brown shrimp were affected, and mortalities approached 100%. In this and other reports the importance of physical or chemical stress to the onset of disease was emphasized.

An epizootic of vibriosis, accompanied by mortalities, was reported in juvenile and adult *P. japonicus* being reared in a heated effluent in southern France (Boemare *et al.*, 1978). *Vibrio parahaemolyticus* and *V. alginolyticus* were principal isolates from moribund animals, and mortality was 100%.

Vibrio spp., as well as members of the genus *Pseudomonas* with chitinolytic capacities, are also responsible for another significant problem in shrimp culture: shell disease (Cook and Lofton, 1973). Epizootics and resultant low but persistent mortalities have been reported in an

experimental shrimp farm by Lightner (1975), who considered the chitinoclastic bacteria (in this case, *V. anguillarum*) to be the primary pathogen.

A wide spectrum of additional diseases has been reported from experimental and commercial shrimp culture in recent years. A fungus, *Lagenidium* sp., is responsible for systemic larval mycosis in penaeid shrimp in the United States (Bland and Amerson, 1973). Another fungus, *Fusarium* sp., infects the gills of juvenile penaeids in Japan and in North America (Egusa and Ueda, 1972; Lightner, 1975). Several epicomensal protozoans (e.g., *Zoothamnium*, *Lagenophrys*, *Epistylis*, *Ephe-lota*) can occur on the gills and the appendages of shrimp and can cause mortalities, apparently by reducing the effective respiratory surface (Couch, 1978). These organisms, as well as infestations of filamentous bacteria (*Leucothrix* spp.), are usually indicators of poor water quality.

Several microsporeans infect wild penaeid shrimp, causing a syndrome known as "cotton," or "milk," shrimp. Infections may occur in epizootic proportions. Recently, successful transmission of microsporidiosis (caused by *Thelohania duorara*, *Thelohania penaei*, and *Pleistophora* sp.) was reported by Iversen and Kelly (1976), using the spotted sea trout, *Cynoscion nebulosus*, a predator of shrimp, as a "transfer," or "conditioning," host for spores. The work needs to be extended, but does suggest that fish predators should be excluded from shrimp ponds and from the vicinity of intake channels. An earlier study (Villella *et al.*, 1970) did not disclose microsporean infections in pond-reared pink shrimp, but the danger of establishment of this group of parasites is real.

C. Lobsters

Lobsters (*Homarus americanus* and *Homarus gammarus*) have yet to be reared commercially, although technological advances in lobster culture have been extensive, as a consequence of economic interest and the existence of a number of research and development projects. Disease is, of course, one of the major areas of attention. A recent review discussed six microbial diseases of cultured lobsters that are of principal concern: (1) shell disease, (2) gaffkemia, (3) microbial epibionts, (4) *Lagenidium* disease, (5) *Haliphthoros* disease, and (6) *Fusarium* disease (Fisher *et al.*, 1978). The first three of these are bacterial, and the last three are fungal.

All life stages of lobsters, including larvae and postlarvae, are susceptible to shell disease, although most studies have emphasized adult lobsters. Death was found to be most frequent in larvae, possibly because of the lack of protection offered by the thin exoskeleton (Fisher *et*

al., 1976a). Adhesions between the shell and the hypodermis prevented molting, and death ensued. Mortality of adults seemed less common, except when extensive areas of the gills were destroyed.

Gaffkemia, caused by the bacterium *Aerococcus viridans* var. *homari*, is known primarily as a disease of juvenile and adult lobsters and can be a particular problem in holding pens, where it may cause extensive mortalities (Goggins and Hurst, 1960). Prevalences in culture facilities have been low, especially when individuals have been separated and water quality has been maintained (Fisher *et al.*, 1978).

Microbial fouling, especially of the gills, has been shown to be lethal to lobster, as well as to shrimp and crab, larvae (Johnson, 1974a; Nilson *et al.*, 1975). Filamentous forms such as the bacterium *Leucothrix mucor* may entangle setae and impair molting. Infestation and destruction of eggs have also been described as causes of mortality (Fisher, 1976).

The fungus *Lagenidium* sp. can infect the eggs and larvae of many crustaceans, including lobsters, shrimp, and crabs (Couch, 1942; Lightner and Fontaine, 1973; Armstrong *et al.*, 1976). Zoospores of the pathogen penetrate the egg membrane or the larval exoskeleton, and the mycelium invades all host tissues, resulting in death.

Another phycomycetous fungus, *Haliphthoros milfordensis*, infects crab eggs, postlarval lobsters, and adult pink shrimp (Fisher *et al.*, 1975; Tharp and Bland, 1977). Invasion occurs at joints or through small wounds; a strong host response results, but mortality occurs frequently as a consequence of tissue destruction or molt impairment, because of adhesions (Fisher *et al.*, 1978).

A third fungal disease, caused by *Fusarium solani*, has been reported in juvenile and adult lobsters, as well as in penaeid shrimp (Johnson, 1974b; Lightner and Fontaine, 1975; Lightner, 1977). Infections produce chronic necrotic lesions of the gills, appendages, and body surface. The disease is progressive; death may be due to toxins produced by the pathogen or to secondary bacterial infections.

This list of six diseases includes the most serious microbial diseases of cultured lobsters—although other diseases have been reported and still others are undoubtedly waiting to make a debut in the future, as lobster culture moves from research and development to the production phase. This list pertains specifically to culture of the American lobster, *H. americanus*. The closely related European species, *H. gammarus*, has similar problems, and a recent review emphasized two current diseases: gaffkemia and fungal (*Fusarium*) disease (Alderman, 1980). Of gaffkemia, Alderman reported: "There have been occasional outbreaks of gaffkemia, which although locally serious for the companies involved, have

not generally led to a widespread problem.” Of the fungal disease, Alderman stated:

In the experimental lobster rearing system at Conwy [Wales], . . . some cases of a fungal disease of the exoskeleton of lobsters were recognised. The disease is caused by a fungus invading the skeleton of the lobster through open wounds. The fungal invasion may eventually be fatal, but it is unlikely to occur frequently except in the type of overcrowded warm water situations which are involved in intensive rearing. Very similar conditions have been noted in lobster rearing systems in the United States. In an article I wrote some 9 years ago, I predicted that this type of fungal disease would become important in enclosed rearing conditions and, with the development of intensive culture of crustacea in tropical and sub-tropical areas, more and more problems of this type have been found. It must, however, be emphasised that this type of problem is likely to be serious only under artificially-intensive rearing conditions.

In concluding this discussion of lobster-rearing problems, it should be pointed out that some of the diseases of cultured lobsters considered here are also found in wild populations (shell disease and gaffkemia, for example), whereas others (such as microbial fouling and some of the fungal diseases) are associated with the hatchery environment and the stresses resulting therefrom.

III. ASSESSMENT OF DISEASE EFFECTS ON CULTURED SHELLFISH

Shellfish pathologists have sometimes been accused of losing touch with reality—of elevating the role of disease in marine aquaculture to unwarranted levels. In fact, at a regional symposium in the United States, a well-informed aquaculturist made the claim that “it was not disease *per se* that was acting as a deterrent to successful culture, but rather the *fear* of disease outbreaks and subsequent economic disaster that was discouraging investment.”

Where is reality here? What has been the experience with species which have been cultured? How important is disease to success or failure? Some of the experience in the United States with marine and anadromous species can be summarized by species or groups of species as follows:

1. *Penaeid shrimp diseases*: Principally because of the extensive research, development, and production activity in the culture of shrimp of the genus *Penaeus*, much information about diseases

and their control has become available. At present, 28 diseases or disease syndromes, including six of viral, three of bacterial, and three of fungal etiology, have been described and seem to constitute reasonable entities, with several others of unknown or uncertain etiology. An apparent problem is the proper recognition of what can be called generalized disease signs, such as black gills, which may result from a number of causes, infectious and noninfectious. Some of the diseases described seem clearly to be related to unsatisfactory water quality.

2. *Malaysian prawn diseases*: Several species of freshwater prawns, particularly the giant Malaysian prawn, *Macrobrachium rosenbergii*, have been the subjects of intensive culture efforts in the United States and elsewhere during the past decade. Because spawning and larval development occur in saline waters, these animals logically become part of marine aquaculture. Thus far, approximately 15 diseases or disease conditions have been described, of which at least three are of infectious origin. Disease problems with *Macrobrachium* culture have been relatively minor, compared with penaeid shrimp culture, and seem to result largely from poor water quality or other stresses characteristic of artificial environments.
3. *Lobster diseases*: Natural production of the American lobster, *H. americanus*, has been unable to meet market demand, despite continuously increasing prices. Several major multidisciplinary research and development projects exist in the United States and in Canada, and much information has accumulated about a number of lobster diseases. Eight have been recognized as being of importance in culture systems—four bacterial, three fungal, and one protozoal—as well as several disease conditions of uncertain etiology.
4. *Blue crab diseases*: Annual production of blue crabs, *Callinectes sapidus*, on the East and Gulf coasts of the United States is high, but variable from year to year. Market demand makes it a desirable species for culture, and some limited attempts at this are being made. The recognized diseases of blue crabs of significance to culture operations have rapidly increased in number. At present, ten diseases—three viral, two bacterial, one fungal, and four protozoal—have been described.
5. *Oyster diseases*: Aquaculture of oysters in the United States emphasizes two species: the American oyster, *Crassostrea virginica*, and the Pacific oyster, *C. gigas*. There is some very limited culture of the Olympia oyster, *Ostrea lurida*, and the European

oyster, *O. edulis*. Oyster hatcheries now exist on both coasts, but their contribution to total commercial production—when compared to natural set—is small. Hatchery diseases recognized so far are viral, bacterial, and fungal and seem to be related to water quality. The most important diseases in grow-out areas are of protozoal etiology.

6. *Clam diseases*: Hatching, rearing, and limited production of hard-shell clams, *Mercenaria mercenaria*, exist on a small scale on the U.S. East Coast. Other species, such as the East Coast surf clam, *Spisula solidissima*, and several West Coast clams, have attracted some research attention, but at present most of the clam production is from natural beds. Only two diseases—bacillary necrosis and larval mycosis—have been clearly associated with larval mortalities, although a number of others have been shown to have deleterious effects.

Microbial agents—viruses and bacteria—can be important causes of disease in shellfish hatcheries, and it might be worthwhile to dwell on these pathogens briefly, since any assessment of disease effects will quickly encounter them.

Information about viral diseases of shellfish was scarce before 1970; in the ensuing years, even without established cell cultures for marine molluscs or crustaceans, the recognition and partial characterization of invertebrate viruses have accelerated remarkably.

Some viruses, probably existing in latent form in wild populations, have emerged as pathogens in culture environments, often as a consequence of stressors such as high temperature, overcrowding, inadequate nutrition, or poor water quality. Examples of information about latent viral infections being disclosed by stress of abnormal environments include an oyster herpes-like viral disease that caused mortalities when oysters were held at high temperatures (Farley *et al.*, 1972), a shrimp baculovirus that caused mortalities in overcrowded holding tanks (Couch, 1974), and a presumed viral disease of clams, disclosed under captive conditions (Brown, 1980).

Among crustaceans, the first viral disease was described by Vago (1966). Since then, a bewildering array of viruses has been described (using electron microscopy) in species such as the blue crab (Johnson, 1983) and shrimp (Sano *et al.*, 1981; Lightner *et al.*, 1983). Some (but not all) of the described viruses have been associated with disease and mortality, in captivity or in culture.

Among marine molluscs, viruses have been associated with disease and mortality of oysters in the United States and in France by Farley *et al.*

(1972) and by Comps and Duthoit (1976). Recently, a presumed iridovirus was identified as the cause of a velar disease in oyster (*C. gigas*) larvae in hatcheries on the west coast of the United States (Elston and Wilkinson, 1985). A more problematic association between virus and hematopoietic neoplasms in clams was made by Oprandy *et al.* (1981).

With the imminent development of marine invertebrate cell cultures, the coming decade should be one of rapid expansion of knowledge about viruses in molluscan and crustacean shellfish, some of which may be of importance in aquaculture.

Looking briefly at bacterial pathogens important in shellfish hatcheries, the genus *Vibrio* is abundantly represented. Some members of the genus can be facultatively pathogenic to stressed animals, and it is this characteristic which is important in the aquaculture environment. At least three species (as usually defined) have been associated with epizootics and mortalities of cultivated animals—vertebrate and invertebrate. These species are *V. anguillarum*, *V. parahaemolyticus*, and *V. alginolyticus*. Vibriosis was and is an important problem in molluscan shellfish hatcheries (Tubiash *et al.*, 1965; Tubiash, 1975; Elston *et al.*, 1982). Epizootics still occur in larval oyster populations (Brown, 1973, 1981; Blogoslawski *et al.*, 1978; Brown and Losee, 1978; Leibovitz, 1978). Vibriosis was also recently implicated by Elston and Lockwood (1983) in mortalities of abalones, *Haliotis rufescens*, on the west coast of the United States. Among crustaceans, vibriosis has been demonstrated to be a persistent problem in shrimp culture—affecting all life stages—and a *Vibrio* sp. was recently isolated from moribund juvenile and adult lobsters in rearing facilities (Bowser *et al.*, 1981). Many *Vibrios* are strongly proteolytic and chitinolytic, as well as halophilic, which probably accounts in part for their success as opportunistic pathogens of marine species in culture.

Agents of microbial diseases in molluscan culture may be characterized as facultative or obligate parasites, with quite different characteristics, as was pointed out by Elston (1984) and as is summarized in Table 15.

Management measures for bacterial diseases in intensive culture systems were also summarized by Elston (1984) as including:

- Recognition of the disease problem
- Identification of the most likely etiology
- Determination and elimination of the source of the infectious agent
- Reduction in the numbers of pathogens in the system

Table 15. Agents of Microbial Diseases of Molluscs—Characterization^a

Facultative or Opportunistic Pathogens	Obligate or Primary Pathogens
May be common in the culture environment at certain times or in certain locations	Often, but not always, highly host specific and tissue specific
Responsible at present for the most significant diseases in intensive molluscan culture, especially in early life stages	Often, but not always, intracellular, and replicate within host cells
May proliferate in culture systems as a consequence of poor sanitation	Often transmitted by movements of animals, introducing stocks with unknown disease history
May proliferate in presence of cultured animals, but uncommon otherwise	May cause significant disease and mortalities in wild, as well as cultured, populations
Vibriosis is at present the most significant disease in intensive culture, followed by infections caused by <i>Pseudomonas/Aeromonas</i>	May produce infection and disease in the absence of other forms of stress
May affect host animals by direct infection or elaboration of toxins, or both	
Often infect animals secondarily, after stress from other environmental factors	

^a After Elston (1984).

- Reduction in stressors (e.g., nonoptimum temperature, salinity, oxygen, water quality)
- Prevention of cross-contamination (e.g., equipment, materials, water supply, animals from outside sources)
- Sterilization of effluents from culture tanks
- Isolation of brood stock facilities from rearing facilities
- Isolation and destruction of infected larval stocks
- Conduct of routine monitoring of influent water for vibrios and other facultative pathogens
- Restriction of antibiotic use to specific crisis situations
- Surface sterilization of juveniles with sodium hypochlorite
- Isolation of new stocks and assurance that they are specific pathogen free before introducing them to the culture facility

Environmental influences on developing shellfish larvae are numerous, and, either singly or in concert, they may affect survival (Fig. 44).

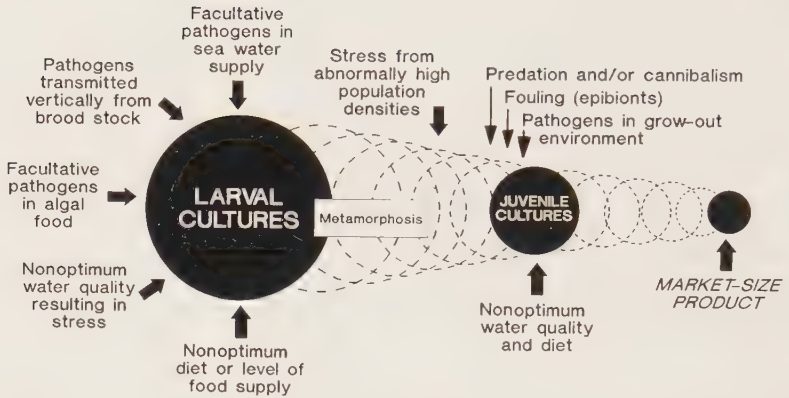


Figure 44. Principal environmental influences on the culture of early life stages of molluscan shellfish. Modified from Elston (1984).

IV. PRINCIPLES AND MECHANISMS OF DISEASE CONTROL IN SHELLFISH CULTURE

In examining the disease control component of managing extensive and intensive shellfish aquaculture systems, it is apparent that some factors are common to both types of systems and some are unique to one or the other (Table 16). A healthy seed stock, adequate nutrition, good water quality, and early recognition and diagnosis of disease problems are control ingredients of universal application in any kind of system. Ex-

Table 16. Principal Elements of a Disease Control Program in Marine Aquaculture

Extensive Culture	Intensive Culture
Healthy seed stock	Healthy seed stock
Adequate nutrition	Adequate nutrition
Prophylactic immunization (fish)	Prophylactic immunization (fish)
Good water quality	Good water quality
Early recognition of a problem and diagnosis of its cause	Early recognition of a problem and diagnosis of its cause
Environmental/stock manipulation	
Control of transfers and introductions	
	Chemoprophylaxis and chemotherapy
	Stress reduction

Table 17. Disease Problems Related to Life History Stages

Culture Phases	Principal Disease Problems
Seed stock (eggs and larvae)	Epizootic microbial diseases of larvae, facultative pathogens enhanced by poor culture conditions
Grow-out (postlarvae)	Epizootics of frank pathogens, nutritional deficiency diseases, chronic diseases producing slow growth and low mortalities
Market sizes	Epizootics of frank pathogens, chronic diseases producing slow growth and low mortalities
Brood stock (spawning adults)	Chronic diseases leading to low continuous mortality, pathogens which may be transmitted vertically through eggs

tensive culture methodology also includes environmental or stock manipulation (moving oysters to low salinities to reduce the effects of particular pathogens, for example) and the necessity for quarantine and disease inspection of nonnative animals being considered for introduction into natural waters. Intensive culture, on the other hand, can take advantage of chemoprophylaxis and chemotherapy not feasible in extensive systems, and must be concerned continuously with stress reduction (in the form of maintenance of adequate water quality, nutrition, and population density in particular).

An important, but often overlooked, truism in the health management of cultured animals is that *the problems and the control strategies may be drastically different at each life history stage*, from egg to adult (Table 17). This is in part because different pathogens may be involved, and in part because the extent of investment in and value of the animal increases with increasing age. Eggs and larvae of many marine species are usually available in quantity, but their *quality* must be maintained by investment in expensive equipment and careful environmental control—otherwise, the entire subsequent history of the cohort may be characterized by lingering difficulties (e.g., slow growth, abnormalities, higher-than-average mortality rates). Poor culture conditions may encourage outbreaks of facultative pathogens such as vibrios in larval populations, often accompanied by extensive mortalities. Frequently, if production schedules can tolerate it, the method of choice in dealing with a “problem batch” of larvae is to discard it and start fresh, since the investment to that point has been relatively low.

Postlarval and juvenile populations may also be subject to epizootics and mass mortalities, but nutritional deficiency diseases and chronic diseases producing low but continuous mortalities emerge as

additional problems. This combination of acute and chronic diseases persists right up to market size.

A new disease factor enters with management of brood stocks: the presence, often in latent form, of infectious diseases which may be transmitted vertically through eggs or horizontally by careless facility practices and hygiene. This can be particularly true for some viral and bacterial problems.

Another truism in health management of cultured animals is that *the battle against diseases is a continuous, long-term one*. Where culture has persisted for long enough—as, for example, in oyster culture—the pattern that has emerged is one of the sequential appearance and spread of new diseases, often accompanied by significant mortalities, until control methods are developed. The new diseases may persist at epizootic levels for many years and then subside to form part of background mortalities, especially if effective control methods are developed. Almost invariably, though, there are additional disease entities, some already enzootic in the population and some introduced from other areas, which may emerge as problems in marine aquaculture. The process appears to be unending and calls for availability of unique expertise in pathology at times of crisis, to insure early diagnosis and development of control measures.

Sometimes, though, even when expertise is available, years of study may be needed to develop understanding of the pathogen, its method of transmission, its life cycle, and its environmental requirements. This has been the case with recent mortalities of oysters in France and in the United States, in which obscure but virulent protistan pathogens have been recognized as etiological agents (Grizel *et al.*, 1974; Ford and Haskin, 1982). Fortunately, even in the absence of adequate scientific information, empirical methods of stock and environmental manipulation can be and have been developed, which allow the industry to survive, even in the presence of epizootic disease. These methods—in the case of the oyster diseases mentioned—include delayed planting of seed until after the infectious period is past (France) or planting seed in low-salinity areas where the pathogen will not multiply (United States).

Viral diseases of Crustacea present a particularly difficult problem. Since aquaculture provides an opportunity for close observation of all life stages of selected aquatic animals, information about their viral diseases has increased enormously in the past decade. This is especially true of shrimp and crabs. It is likely that the viral diseases already recognized, and many others not yet recognized, exist in latent form in populations and may be provoked into patency by stresses of the culture environment (e.g., abnormal temperatures, low oxygen levels, or high

population densities). Control of viral diseases is difficult, so it is particularly important that the etiological agents not be introduced into cultured populations or culture facilities from infected sources (for an example of the potential consequences of such actions, see Lightner *et al.*, 1983). Control measures at present consist principally of viral eradication by the destruction of infected stocks and sterilization of the facilities.

Beyond these generalizations about disease control, it might be important to outline and discuss a specific seven-point control program, which should be a foundation for economically viable shellfish culture. Points to be considered are:

1. Diagnostic services
2. Stress and stress management
3. Vaccination and enhancement of disease resistance
4. Chemotherapy
5. Regulation of transfers and introductions
6. Stock manipulation
7. Environmental manipulation

These seven points can form an integrated program which will do much to reduce disease effects on shellfish populations.

A. Diagnostic Services

Adequate diagnostic services must be available to aquaculture entities, regardless of the kind of culture—extensive or intensive. Mortalities above background levels reduce economic viability; mass mortalities can eliminate entire crops, and, if repeated, can lead to bankruptcy. *Remedial measures must be based on rapid and correct diagnosis of the cause of mortalities—or, better still, on the detection of incipient problems*, whether they be disease or some other abnormal condition, which could lead to mortalities. Often, nonoptimum culture conditions can be detected by the appearance of epibionts or facultative pathogens or by slow growth and abnormal behavior.

Diagnosis of disease requires a high level of expertise, not usually affordable by individual aquaculture operations. Such expertise is sometimes available from government-supported (federal or state) laboratories or programs designed to provide diagnostic services to industry, or from private consultants or laboratories which provide diagnostic services at a price. Whatever the source of diagnostic help, response time must be short; usually, by the time mortalities begin in culture systems, it is too late to save most of the affected population, and every hour of delay reduces the percentage of survivors.

The reality is that the staff of the aquaculture operation must take primary responsibility for observing and reporting any abnormality that may signal a potential disease problem. The facility manager must then decide on a course of action, based largely on previous experience and on advice from any immediately available sources. Sometimes a preliminary and tentative diagnosis can be made by an aquaculture disease specialist during a phone conversation, although this can be a risky approach. A site visit and examination of appropriate samples (i.e., tissues and cultures) are much better bases for diagnosis, if they can be carried out promptly. A simultaneous examination of other environmental factors in the culture system—chemical and physical—should be done. Too often, in examining aquaculture mortality problems, focus is narrowed to a search for pathogens, when the real cause may be environmental, nutritional, or physiological, sometimes with the superimposition of infections by facultative microorganisms in weakened or damaged hosts.

B. Stress and Stress Management

Stress management as a prophylactic method is not a new concept; concern about the effects of poor water quality, overcrowding, inadequate diet, and abnormal temperatures or salinities has long been a feature of aquaculture practice. Among the many aspects of stress which are relevant to captive marine populations are these:

1. Chronic marginal physical/chemical conditions, commonly called poor water quality, can be important determinants of mortality.
2. Facultative pathogens, especially vibrios and pseudomonads, are particularly effective when individuals are stressed.
3. Latent infections, introduced with hosts to aquaculture environments, can become patent in stressed populations.
4. Even though concepts of physiological stress were developed for vertebrates, it is becoming apparent that invertebrate stress responses exist and have been described for several species. Some stress management activities for intensive aquaculture are illustrated in Fig. 45.

Stress is a significant but elusive concept in biology, and, like any concept, it can be misunderstood, misinterpreted, or abused. In its original sense, as developed by Selye (1950, 1955, 1976), stress represents the consequences of all of the mechanisms whereby an organism attempts to maintain equilibrium in the face of environmental change. Perspec-

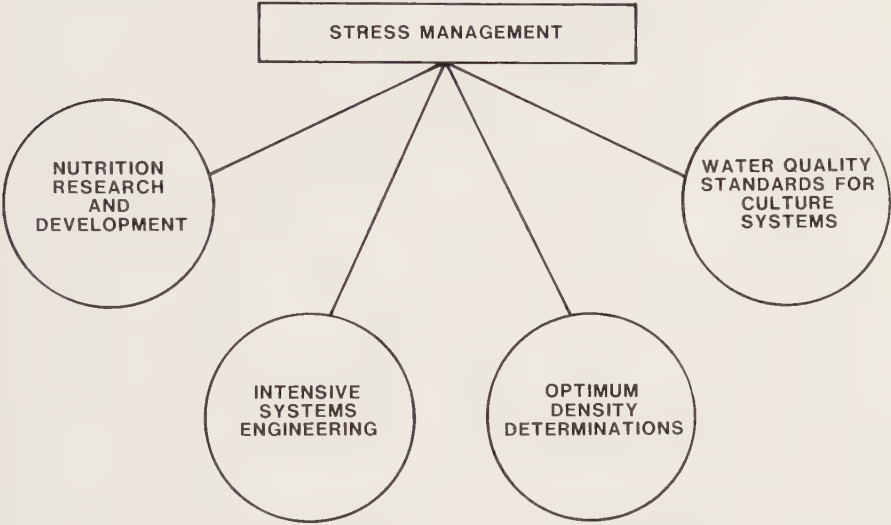


Figure 45. Principal components of a stress management program for intensive marine aquaculture.

tives on stress will vary with the background of the observer. To the pathologist, many of the responses to stressors can be described as, or result in, “disease” if disease is defined as “any departure from normal structure or function of the animal.” Specific responses to stressors, from the viewpoint of the pathologist, include (in probable descending order of priority): (1) the classical cell and tissue changes, such as inflammation (acute and chronic), degeneration (including edema, necrosis, and metaplasia), repair and regeneration (proliferation, hyperplasia, and scar formation), neoplasia (including consideration of cell origin, stage, and type—whether benign or malignant), and genetic derangement (including chromosomal changes, and some skeletal abnormalities); (2) changes in resistance to infection; and (3) physiological, biochemical, and behavioral changes. *One of the most important consequences of stress is increased susceptibility to disease;* in fact, disease in aquaculture populations is usually an indication of marginal or poor environmental conditions. This is especially true of diseases caused by facultative pathogens such as vibrios, pseudomonads, and aeromonads.

Although most studies of the physiological and morphological consequences of stress have emphasized vertebrates, there are indications that counterpart phenomena may exist in invertebrates as well. Among Mollusca, several publications have provided evidence for a generalized response syndrome. Jeffries (1972), working with clams, *Mercenaria mer-*

cenaria, found a shortened life span, mass mortalities, a reduction in total amino acid and carbohydrate content, and shell invasion by *Polydora* in stressed clams. He considered amino acid ratios, particularly the taurine/glycine ratio in gill and mantle tissues, as the best indicators. Ratios above 3 indicate stress, with ratios above 5 indicating acute stress. High taurine ratios were also observed by Feng *et al.* (1970) in oysters infected with the trematode *Bucephalus*. In Jeffries' study, the exposure of clams to petroleum resulted in black tar-like concretions in amoebocytes and kidney tissues. The concretions collected in the renal sac and plugged the kidney tubules.

Other indices of stress in molluscs have been proposed—particularly reduced "scope for growth" (a measure of the potential for somatic growth and gamete production) (Bayne, 1975; Bayne *et al.*, 1979) and the oxygen consumption/nitrogen excretion ratios (Corner and Cowry, 1968; Bayne and Scullard, 1977). Working with mussels, Bayne and associates observed a decline in condition and depression of gametogenic activity under stress conditions. Other studies by Moore (1976, 1977) related the degree of cell damage due to destabilization of lysosomal membranes to degrees of stress.

Among the larger Crustacea, increased levels of hemolymph glucose have been recognized as responses to a variety of stressors (Telford, 1974). A clinical method for the detection of changes in hemolymph glucose as a measure of stress in the pink shrimp, *Penaeus duorarum*, was proposed by Smith (1983).

An important aspect of stress management in marine aquaculture is early recognition of abnormality. In bivalve molluscs signs of stress may include mantle recession, pale digestive gland, regression of digestive tubule epithelium, hemocyte infiltration of tissues, edema, lag in gametogenesis, and increased ceroid (brown bodies)—all or most of which constitute a stress syndrome.

In crustaceans signs of stress include black gills, abdominal muscle opacity, molt retardation, exoskeletal overgrowth with filamentous bacteria and protozoan epibionts, the frequent occurrence of shell disease, disoriented behavior, the presence in the tissues of gram-positive bacteria, and clotting of the hemolymph (as a response to gram-negative endotoxin)—again constituting a stress syndrome.

C. Vaccination and Enhancement of Disease Resistance

Invertebrates do not respond to the presence of foreign proteins by production of specific immunoglobulin antibodies comparable to those of vertebrates. This reality has limited attempts to develop vaccination

programs to enhance disease resistance in shellfish populations, but there is some information suggesting the presence of less specific responses to infection in invertebrates (Bang, 1975). Recent work with American lobsters (Paterson and Stewart, 1974; Schapiro and Steenbergen, 1974; Schapiro *et al.*, 1974) has demonstrated increased phagocytic activity, with a suggestion that vaccines prepared from avirulent strains of the pathogen *Aerococcus viridans* produced a limited degree of resistance to the disease (gaffkemia) which it causes. Additionally, injections of antibiotic (vancomycin) plus virulent bacteria provided short-term protection, as did injection of heat-killed pathogens (Arie and Stewart, 1984). In other studies, increased bactericidal activity, following the injection of a bacterial antigen, was found to exist in spiny lobsters (Acton *et al.*, 1969).

Certainly, more work needs to be done, especially with the larger Crustacea, on the enhancement of individual resistance to disease. Prophylactic enhancement of nonspecific resistance—possibly by exposure of postlarvae to killed or attenuated pathogens—may prove to be the crustacean counterpart of prophylactic immunization in fish. It seems more likely, though, that a more appropriate research approach for invertebrates generally is that of *enhanced population resistance by genetic selection*. Several examples of the likelihood of success of this approach exist. The development of disease resistance in populations of oysters exposed to epizootic levels of particular pathogens is suggested by recent observations. A high degree of natural selection for disease resistance in oyster populations exposed to the protozoan *Haplosporidium nelsoni* has been reported by Andrews and Frierman (1974). An unknown pathogen responsible for extensive oyster mortalities (1916–1959) in the Gulf of St. Lawrence, Canada, has also resulted in selection for disease resistance, in that oysters from other areas die when introduced, whereas native oysters do not die at present.

So we now have several examples in invertebrates (oysters) where severe natural selection during epizootics seems to have produced resistance in surviving populations. Attempts are now under way at several research institutions in the United States to enhance resistance experimentally in oysters, and the objective of producing resistant stocks is a logical one for any genetic modification program with controlled reproduction.

D. Chemotherapy

Even though the best approach to disease control emphasizes stress reduction in aquaculture facilities, there are occasions when chemotherapy can be a control method of choice, and facility managers are most

content when an armamentarium of chemical remedies is available on the shelf. Recognizing these facts of existence in aquaculture production, chemotherapeutic methods have been developed to treat some of the diseases encountered. Some of the microbial diseases can be treated successfully, especially if treatment is instituted early, before mortalities begin. A number of the chemicals found to be effective in fish culture, such as malachite green, are also effective in crustacean culture, and applications have been adapted for use with invertebrates.

Chemotherapy has been employed successfully in the treatment of some larval diseases of lobsters and other crustaceans (Fisher and Nelson, 1977; Fisher *et al.*, 1978). Larvae with shell disease have been reared successfully by dipping them in 20 ppm malachite green for 8 minutes every other day during the larval period (Fisher *et al.*, 1976b). This regimen, combined with rigorous selective culling and adequate nutrition, retarded spread of the disease and reduced its prevalence.

Gaffkemia in lobsters, caused by the bacterium *A. viridans* var. *homari*, has been responsive to injections of the antibiotics vancomycin and penicillin (Stewart and Arie, 1974; Fisher *et al.*, 1978). In one study, the bacterium was eliminated by injection of 20,000 units of penicillin per 500 g of body weight.

Control of epibiotic fouling of lobster larvae can be effected by chemotherapy, even though improved facility hygiene and stress reduction are methods of choice. In one study continuous addition of low concentrations of streptomycin and penicillin increased egg and larval survival, and malachite green also inhibited bacterial and algal epibionts (Fisher *et al.*, 1978). In another study, with shrimp larvae, quinacrine hydrochloride (0.6 mg/liter) and other quinine compounds were found to be effective against infestations of the stalked ciliate *Epistylis* sp. (Johnson, 1976).

Several fungal diseases can affect crustacean eggs and larvae and can cause extensive mortalities if unchecked. One, caused by the phycomycete *Lagenidium* sp., has been treated successfully by dipping larvae in malachite green (Bland *et al.*, 1976; Fisher *et al.*, 1976b). It was noted, however, that antibiotics used to control microbial epibionts may drastically increase the pathogenicity of the fungus (D'Agostino, 1975; Nilson *et al.*, 1976). It was also found that zoospore production could be limited by application of the fungicides trifluralin and captan (Armstrong *et al.*, 1976). A second phycomycetous fungus, *Haliphthoros milfordensis*, was treated effectively in cultures of postlarval European lobsters by malachite green and furanace, although other chemicals, such as crystal violet, acriflavine, and methylene blue, were of some therapeutic value (Abrahams and Brown, 1977). A third fungal disease

of lobsters and shrimp, caused by *Fusarium solani*, proved resistant to practical chemotherapy, although several fungicides showed promise in *in vitro* studies (Hatai *et al.*, 1974).

Chemotherapeutic methods of disease control have been applied to molluscan culture as well, although not as extensively as to crustacean culture. One recent and somewhat unique application of chemotherapy (using ozone and "Bactipal") was reported by French researchers. In a search for methods to reduce the effects of the protistan parasite *Bonamia ostreae* on flat oysters, *Ostrea edulis*, infected oysters and effluents of holding basins were treated. In preliminary experiments Bactipal was lethal to the parasite at 1 ppt for 30 minutes, and ozone was effective at 3.5 mg/liter for 60 minutes, (H. Grizel, unpublished observations, 1983).

Chemicals added to intensive culture systems for disease control may perform two important positive functions: they may reduce or eliminate pathogens and they may reduce or control populations of heterotrophic microorganisms which may act as facultative pathogens of animals under stress. Such chemicals may, on the other hand, cause problems in culture systems: (1) they may have negative effects on biological filters in controlled recirculated systems—particularly on nitrifying bacteria; (2) they may have negative side effects on algal food, or on algae present in fish larval rearing tanks; and (3) they may leave undesirable or harmful residues in cultured animals.

With all of their limitations, chemoprophylaxis and chemotherapy still occupy significant niches for response to crises or otherwise intractable problems, and profit-oriented aquaculture producers are most comfortable when "remedies" are at hand.

E. Regulation of Transfers and Introductions

Aquaculture has been a principal motivating force for the movement of shellfish from one geographic area to another—even from one continent to another. One possible consequence of such transfers and introductions is the insertion of pathogens into new host populations in recipient areas. Within the past decade there has been growing national and international concern about the possible consequences of such movements. Natural resource managers, faced with the necessity to make decisions about shipments of fish and shellfish destined for waters under their control, have been sensitized to the possibility of spreading diseases, and have been forced into risk assessments. International organizations, such as the International Council for the Exploration of the Sea (ICES), have developed codes of standard practices, by which national authorities can make decisions about international shipments on some rational scientific basis, rather than on a purely economic one.

At present, treatments for many shellfish diseases are unavailable. This is particularly the case for viral and protozoan diseases. Because of this, limiting the spread of pathogens through aquaculture practices is of great importance. Quarantine and inspection procedures and politically insensitive policies and regulations about importation of live animals can be major deterrents to the dissemination of pathogens.

Exclusionary regulations should not, however, be too inflexible. Transfers and introductions for aquaculture purposes should be feasible, once adequate study has been made and risks have been assessed. The use of standard inspection protocols, possible quarantine provisions, and limited initial quantities of imports all provide a measure of assurance of reduced danger from the spread of disease.

In introducing a new species for aquaculture purposes, several disease scenarios may prevail: (1) diseases may be carried with imports and spread to native stocks, (2) diseases of native stocks may spread to introduced stocks, or (3) hybrids of native and introduced species may have less resistance to particular diseases.

Several examples of these consequences exist. Imports of Pacific oysters, *Crassostrea gigas*, to France and subsequent hybridization with local Portuguese oysters, *C. angulata*, resulted (it is postulated) in the reduced resistance of the hybrids to the pathogen *Bonamia ostreae*. Also, introduction of the parasitic copepod, *Mytilicola orientalis*, to the west coast of North America with imports of its host, *C. gigas*, resulted in the spread of the parasite to native oysters, *Ostrea lurida*, and mussels, *Mytilus edulis*.

An emerging principle concerning introduced stocks of shellfish is that the diseases which kill imported stocks are not usually those known in home waters, but they are not necessarily diseases known in native stocks of the importing country either. An example would be Denman Island disease or microcell disease of *C. gigas* in Canadian Pacific waters, which is unknown in native stocks in Japan. It is possible, of course, that some of these diseases may be latent in either native or imported stocks.

An extreme position about the severe diseases of oysters has been expressed: that the pathogens were *introduced*, either by transfer of resistant members of the same or different oyster species from enzootic areas or by movement of reservoir or alternate hosts. According to this suggestion, all major outbreaks of severe pathogens are the consequences of introductions or transfers.

Transfers and introductions of species, from one hydrographic regimen to another, or from one ocean to another, have characterized the development of marine aquaculture. Such activities are accelerating, and the risk of introduced disease is always present. Some attention is being given to that risk, but adequately documented examples of disease intro-

ductions are rare so far for marine species. Specific illustrations include the introduction of a parasitic copepod (*M. orientalis*) from Japan to the west coast of the United States with introduced Pacific oysters (*C. gigas*) and the introduction of a viral disease to Hawaii (and possibly elsewhere) with introductions of penaeid shrimp from Central and South America (Lightner *et al.*, 1983). The shrimp virus, called infectious hypodermal and hematopoietic necrosis virus (IHHNV), is highly lethal to *Penaeus stylirostris* and infective to *Penaeus vannamei* and *Penaeus monodon*. It has apparently been widely distributed throughout the world with uncontrolled introductions, for aquaculture purposes, of infected *P. stylirostris* or *P. vannamei* from the west coast of Central and South America. Its potential infectivity to other commercial penaeid species is as yet undetermined, but an obvious hazard exists. It may well prove to be the best example to date of widespread dissemination of a pathogen with introduced aquaculture species.

Transfers and introductions of nonnative species are increasing, and some (such as the movement of oyster seed from Japan to the United States Pacific coast and to France) are now considered part of current commercial practice. The parasitic copepod, *M. orientalis*, was introduced to the United States with the early Japanese imports; it proved infective to native West Coast oysters, *O. lurida*, and may have contributed to the decline of that species. More recently, *M. orientalis* was observed in mussels, *Mytilus edulis*, from the principal producing areas on the northern French coast. The parasite was probably introduced with massive imports of Pacific oysters, *C. gigas*, from Japan and the west coast of North America.

In addition to the well-known copepod parasite, two disease entities have been found in Japanese parent stocks. One, a bacterium that causes what has been called multiple abscesses or focal necrosis, has been found in up to 20% of samples of adult oysters from Japan. Seed oysters imported from Japan were found to be affected by this same disease, and the condition was also detected in adult oysters grown on the Washington coast. A second disease, caused by an amoeboid organism of undetermined taxonomic position, was found in 1966 in over 30% of oysters from an area of Matsushima Bay, Japan, examined at the Oxford (Maryland) Laboratory of the National Marine Fisheries Service. Oyster mortalities in this area of Japan had been high since 1960. What may be a similar amoeboid organism has also been recognized in oysters grown in Humboldt Bay, California, from Japanese seed exported from Matsushima Bay (Becker and Pauley, 1968). Annual mortalities in Humboldt Bay were high during the 1960s, and a dredged sample in which 50% of the oysters had died recently (as indicated by the persistence of two shell valves joined by the hinge ligament, and by unfouled inner

shell surfaces) was examined by the author in the summer of 1966. Lesser mortalities of undetermined origin have occurred in other West Coast growing areas.

We know, then, of three disease agents that are present in U.S. West Coast oyster stocks: the parasitic copepod, the multiple abscess bacterium, and the amoeboid organism. All can cause pathological changes in oysters, and all apparently have been introduced from Japan. They, like a number of important shellfish predators, such as Japanese oyster drills and carnivorous flatworms, are now in U.S. waters and may affect native as well as introduced populations. It should be reemphasized, however, that, as yet, no clear association of specific pathogens with mortalities of *C. gigas* has been established on the Pacific coast of the United States.

The oyster stocks of the North American East Coast have also been severely affected by disease-associated mass mortalities during the past 40 years. Four principal disease entities exist: Delaware Bay disease, caused by the ascetosporean *Haplosporidium nelsoni*; seaside disease, caused by the ascetosporean *Haplosporidium costale*; "Dermo" disease, caused by the api-complexan *Perkinsus marinus*; and Malpeque Bay disease, caused by an unknown pathogen. All are apparently distinct from diseases seen in Pacific oysters (Fig. 46). No significant quantities of Pacific oysters have been introduced commercially on the East Coast, nor has there been mass importation of foreign seed comparable to introductions made on the West Coast. Different diseases seem to dominate in particular geographical areas on the East Coast. There is and has been appreciable transfer of oysters from one geographical area of the coast to another, which may well have enhanced the spread of certain of these diseases.

Faced as we are with transfers and introductions—at present under very loose control, if any, depending on state regulations—the proper course of action is difficult to prescribe. The obvious "hard-line" action would be strict quarantine and exclusion of imports. A more feasible approach would be strictly controlled introductions, but this method requires an extensive and expensive inspection and testing organization, probably on a federal level. Inspection could be combined with the restriction of introduced exotic species to particular localities or, better still, to closed systems of culture, using only offspring for introductions in noncontaminated areas. In this way predators and most diseases could be excluded. It is difficult, however, to screen introductions for all possible pathogens, particularly the viruses. Cryptic life cycle stages of known pathogens may exist, and organisms pathogenic to exotic species in their natural habitats may be poorly understood. Also, organisms which are not serious pathogens for the introduced species because of



Figure 46. Areas of the Atlantic coast of North America where disease-caused oyster mortalities have occurred: (A) Malpeque Bay Disease in the Gulf of St. Lawrence, caused by an unknown pathogen; (B) Seaside Disease on the coasts of Virginia and Maryland, caused by the ascetosporean *Haplosporidium costale*; (C) Delaware Bay Disease on the coasts of the Middle Atlantic States, caused by the ascetosporean *Minchinia nelsoni*; and (D) "Dermo" Disease of the South Atlantic and Gulf coasts, caused by the apicomplexan *Perkinsus marinus*.

long association and natural immunity may have severe effects on native species in the area of introduction. On the other hand, pathogens with low infectivity may require large or repeated introductions to become established in a new area; or pathogens may, even after repeated introduction, remain at insignificant levels because of other limiting environmental factors. Often, the course of events is difficult to predict, although experimentation may help to define the level of risk involved.

It is difficult to make direct associations of introduced species with the occurrence of a new pathogen in native populations of the recipient country. Suspected but unproved disease introductions include a viral gill disease of oysters (*C. angulata*) in France (Comps *et al.*, 1980) and a protozoan disease of flat oysters (*O. edulis*), also in France (and more recently in other European countries) (Pichot *et al.*, 1981). In the recent French oyster epizootics, pathogens were identified (an iridovirus re-

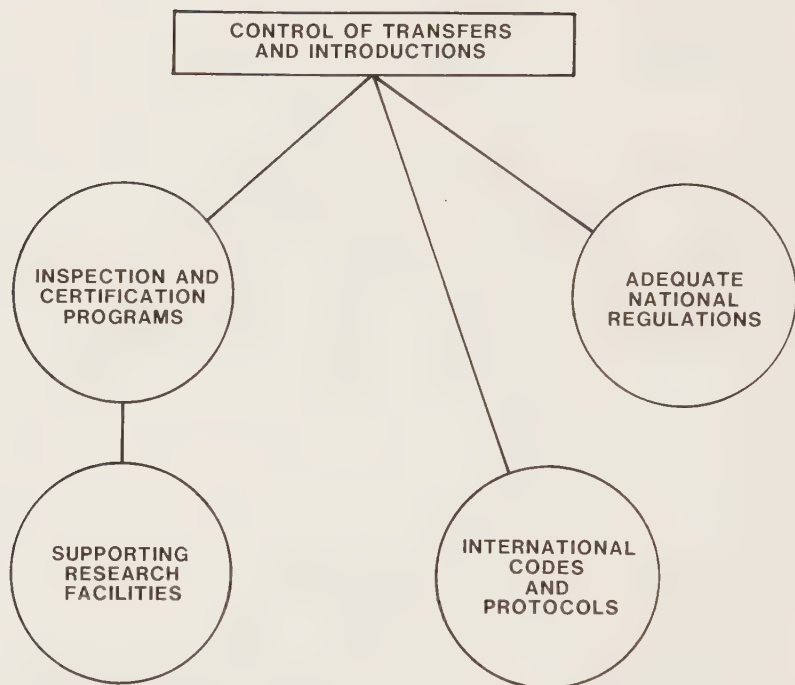


Figure 47. Important components of a program to reduce risks associated with the introduction of nonindigenous species.

sponsible for the gill disease of Portuguese oysters, *C. angulata*, and the haplosporidan *Bonamia ostreae*, responsible for mortalities of *O. edulis*) which have counterparts in oysters from elsewhere in the world and may have been introduced to France recently with massive importations of Pacific oysters (*C. gigas*). No real evidence exists to support this speculation, but there is a cadre of knowledgeable people who feel that many, if not most, of the epizootics in shellfish populations are the consequences of introductions of exotic pathogens. Important components of a program to control introductions are diagrammed in Fig. 47.

There is a general recognition among intergovernmental groups in the Western Hemisphere (e.g., ICES, the European Inland Fisheries Advisory Commission (EIFAC) of the Food and Agriculture Organization of the United Nations) of the need for adequate inspection, certification, and control programs to reduce risks associated with introductions of aquatic species. This recognition has led to a proposal by the International Council for the Exploration of the Sea (ICES) for an international code of practice, which is reproduced here because of its limited availability in the published literature.

*Revised Code of Practice to Reduce the Risks of Adverse Effects
Arising from Introduction of Marine Species*

At its Statutory Meeting in 1973 the ICES adopted a "Code of Practice to Reduce the Risks of Adverse Effects Arising from Introduction of Non-indigenous Marine Species." At its Statutory Meeting in 1979 the Council adopted a revised code, as follows:

1. Recommended procedure for all species prior to reaching a decision regarding new introductions (a recommended procedure for introduced or transferred species which are part of current commercial practice is given in Section 4).
 - a. Member countries contemplating any new introduction should be requested to present to the Council at an early stage information on the species, stage in the life cycle, area of origin, proposed place of introduction and objectives, with such information on its habitat, epifauna, associated organisms, potential competition to species in the new environment, etc., as is available. The Council should then consider the possible outcome of the introduction, and offer advice on the acceptability of the choice.
 - b. Appropriate authorities of the importing country (including fishery management authorities) should examine each "candidate for admission" in its natural environment, to assess the justification for the introduction, its relationship with other members of the ecosystem, and the role played by parasites and diseases.
 - c. The probable effects of an introduced species in the new area should be assessed carefully, including examination of the effects of any previous introductions of this or similar species in other areas.
 - d. Results of (b) and (c) should be communicated to the Council for evaluation and comment.
2. If the decision is taken to proceed with the introduction, the following action is recommended:
 - a. A brood stock should be established in an approved quarantine situation. The first generation progeny of the introduced species can be transplanted to the natural environment if no diseases or parasites become evident, but not the original import. The quarantine period will be used to provide opportunity for observation for disease and parasites. In the case of fish, brood stocks should be developed from stocks imported as eggs or juveniles, to allow sufficient time for observation in quarantine.

- b. All effluents from hatcheries or establishments used for quarantine purposes should be sterilized in an approved manner.
 - c. A continuing study should be made of the introduced species in its new environment, and progress reports submitted to the International Council for the Exploration of the Sea.
 3. Regulatory agencies of all member countries are encouraged to use the strongest possible measures to prevent unauthorized or unapproved introductions.
 4. Recommended procedure for introduced or transferred species which are part of current commercial practice.
 - a. Periodic inspection (including microbiological examination) by the receiving country of material prior to mass transplantation to confirm freedom from introducible pests and diseases. If inspection reveals any undesirable development, importation must be immediately discontinued. Findings and remedial actions should be reported to the International Council for the Exploration of the Sea.
 - b. Inspection and control of each consignment on arrival.
 - c. Quarantining or disinfection whenever possible and where appropriate.
 - d. Establishment of brood stock certified free of specific pathogens.

It is appreciated that countries will have different attitudes toward the selection of the place of inspection and control of the consignment, either in the country of origin or in the country of receipt.

A schematic plan for introductions, following the Code of Practice, is presented as Fig. 48.

F. Stock Manipulation

Extensive culture systems, in which animals are released into the natural environment for growth to market size, are less amenable to control measures than are intensive systems. Some of the elements already discussed are still applicable to extensive systems. Stress management may consist of controlled density planting of oysters or selecting grow-out areas away from known sources of industrial pollution. Control of introductions of nonindigenous species to open waters is a critical element in extensive culture. In addition, stock manipulation may emerge as the most important disease control element in extensive aquaculture.

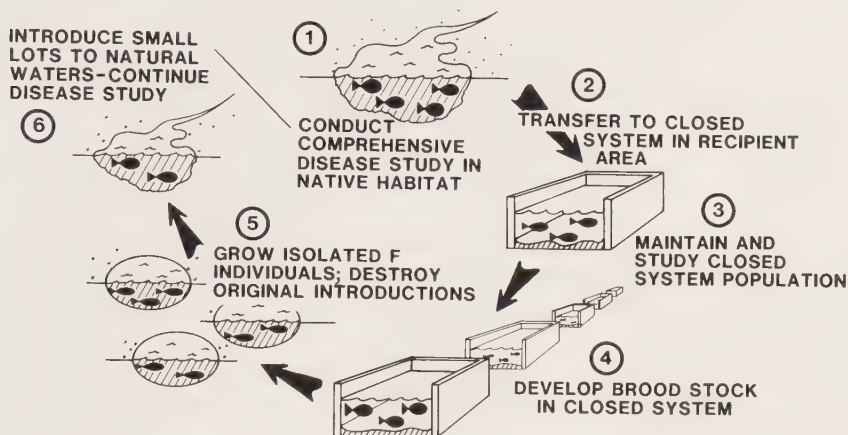


Figure 48. Steps in the introduction of a new species, following the International Council for the Exploration of the Sea's Code of Practice.

systems. Stock management and planting of oysters in the presence of pathogens is a good example. In the case of the protozoan pathogen *Perkinsus marinus*, low-density planting of oysters reduces the risk of disease transmission; in the case of the haplosporidan pathogen *Haplosporidium nelsoni*, planting in waters with salinities below 15 ppt is critical to survival; in the case of the French oyster pathogen *Marteilia refringens*, infections occur in July and August, so seed oysters are not planted during this period. These pragmatic management approaches, utilizing available physiological and ecological information about the pathogen and the host, can insure the maintenance of production even in the presence of serious pathogens.

The disease of planted American oysters, caused by the protozoan *P. marinus*, can serve as an excellent example of the use of stock manipulation methods to limit effects. Infections are greatly influenced by the proximity of susceptible hosts. Mackin (1962) speculated that epizootics may have followed initiation of the practice of concentrating oyster plantings in small areas. It was his opinion that "This one factor has made those areas where crowded planting is the rule the areas of most intense development of oyster disease [Louisiana and Virginia]." He asked the question, "Has the modern planting method itself precipitated the most acute crisis in oyster production ever observed?" His summary suggested the answer: "Part of the increasing toll of disease may be due to heavily crowded plantings, constant re-introduction of large numbers of susceptible oysters to areas of endemic disease, and repeated re-introduction of disease by exotic oyster populations."

The reduced effects of *P. marinus* disease during periods of lower water temperatures and in lower salinities have important implications for oyster culture in enzootic areas. The disease is active for most of the year in the Gulf of Mexico, but is quiescent for almost half the year in Chesapeake Bay. Ray and Chandler (1955) found that in Louisiana the entire crop of oysters could be lost in one summer because of the pathogen. Andrews and Hewatt (1957) reported that oysters could be held for two or three summers in Virginia before yield was reduced by the disease to a level which returned no profit. Losses could be minimized by limiting the number of summers that oysters were held in enzootic areas and by maximum use of low-salinity areas.

Age and size of the oysters influence the prevalence of *P. marinus* infections and the mortality rate. Spat are thought to be refractory to infection until they are 3–4 months old, and death rates are low during the first year of life. Prevalence and mortality rate both increase with age and size. Thus, it is important to plant the largest seed possible, and to harvest oysters as soon as they reach market size. To minimize losses due to *P. marinus*, Andrews and Hewatt (1957) and Andrews (1979) recommended planting in early autumn, to take advantage of autumn and spring growth before infections occur. Harvesting in late spring was also advised.

Variations in susceptibility to the protozoan pathogen, pointed out by Andrews and Hewatt (1957), have important implications for oyster planters. As an example, oyster seed from seaside bays of Virginia was so susceptible that its use in enzootic areas was precluded if more than a single summer's exposure to the pathogen was required. Comparison of seed from several states disclosed that the South Carolina stocks were the least susceptible of those tested.

G. Environmental Manipulation

Environmental management of the culture system is undoubtedly as important as the other disease control methods already discussed (Fig. 49). Disease control should be more feasible in controlled (intensive) culture than in open (extensive) culture, since under controlled conditions it is possible to manipulate water temperature and quality, to observe early indications of disease, and to eliminate parasites with complex life cycles. However, in controlled intensive culture animals must be crowded and fed on artificial diets, which may result in deficiency diseases. Also, bacterial populations in the system must be monitored closely.

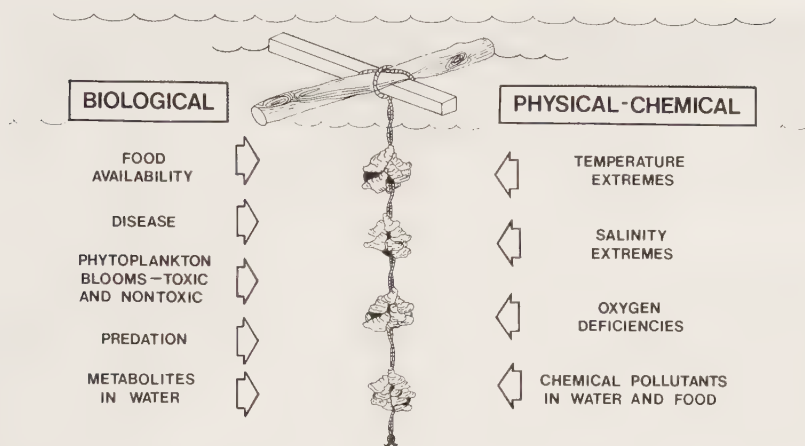


Figure 49. Principal environmental factors operative in aquaculture systems.

Effects of pollution, whether resulting from a buildup of toxic metabolic products within the culture system or from introduction of contaminants from outside the system, can be severe. Stress from pollutants may be expressed in a number of ways, including diminished reproductive activities; damage to genetic material of the egg or embryo, with resulting mortality or abnormal development; direct chemical damage to cell membranes or tissues; modification of physiological and biochemical reactions; changes in behavior, often due to chemical damage to sensory organs; increased infection pressure from facultative microbial pathogens; and reduced resistance to infection. Sublethal effects, such as spawning failure, poor survival of larvae, larval setting failure in the case of molluscs, reduced growth rates, and increased vulnerability to other environmental limiting factors, can have significant effects that may be less apparent. Greater understanding of such sublethal effects is needed to assess fully the influence of any single stress factor on aquaculture production.

Early life history stages of marine animals are usually (but not always) more susceptible to chemical stress than later stages. Effects may be obvious, resulting in death, or less obvious—such as genetic damage, which may not be expressed until later developmental stages (as abnormalities in structure or function). Genetic damage to sex products, embryos, or larvae may be an important and as yet poorly understood consequence of chemical contamination of culture waters.

An important component of the management of cultivated populations is, of course, adequate nutrition. Nutritional deficiency diseases

have received much attention in freshwater fish hatcheries, and reasonably adequate diets have been developed by trial and error over decades. Much of marine crustacean aquaculture is still hampered by the absence of complete artificial diets, and several deficiency diseases have been recognized. With penaeid shrimp, for example, ascorbic acid-deficient diets result in weakening and mortality of juveniles in a syndrome called "black death" (Lightner, 1977).

With this seven-point program of disease control firmly in place, some measure of command over outbreaks in aquaculture becomes feasible. The full program is expensive, though, and must be modulated carefully for cost effectiveness. If production costs, with the added burden of disease control, become excessive, then the practical aquaculturist will look for alternate solutions—and there are some—such as

- "Farming around" existing diseases, accepting annual losses if they are not too destructive, and modifying culture practices to minimize disease effects
- Propagating resistant stocks through a program of subsidized shell planting and creation of spawning stock reserves
- Introducing resistant alternate species, much as the French have done with the Pacific oyster, *Crassostrea gigas*
- "Waiting out" the epizootic, reducing culture operations to a minimum until population resistance increases as a consequence of mortalities

Looking at the actuality—at the pragmatic steps that have been taken in the past by the shellfish industry to enable survival in the face of the continuing presence of epizootic disease—the following examples exist:

- Continuing epizootic levels of the protozoan pathogen *Perkinsus marinus* in oysters, *C. virginica*, of the Gulf of Mexico and South Atlantic states of the United States has led to practices which can be described as "farming around" the problem. Prevalences and mortality rates increase with age and size, so large seed oysters are planted, and beds are harvested as soon as oysters reach minimum market size. Furthermore, maximum use is made of low-salinity areas, oysters are planted thinly on beds (to reduce direct transmission), and the numbers of summers (the infective period) that oysters are held in enzootic areas are limited (Andrews, 1976).

- Diminished production of the Portuguese oyster, *C. angulata*, on the coast of France in the late 1960s and the early 1970s, due in part to a viral gill disease, led to a decision to import large quantities of a more resistant substitute species, the Pacific oyster, *C. gigas*, from Japan and the west coast of North America. Imports consisted of seed and adult oysters and continued for several years.
- Mortalities of oysters, *C. virginica*, on the north coast of New Brunswick, Canada, in the mid-1950s, due apparently to Malpeque Bay disease of unknown etiology, resulted in mass transfers of oysters from Prince Edward Island, which were considered resistant to the disease (Logie, 1956; Alderman, 1980), since they were offspring of survivors of mortalities which had begun on the Island in 1915.

Each of these approaches seemed effective, insofar as the continued existence of the industry could be used as a criterion. Oyster production in the southeastern United States has been maintained, despite the effects of *P. marinus*; the Portuguese oyster has been almost entirely replaced on the French coast by the Pacific oyster, and production now exceeds 100,000 tons; and oyster production on the New Brunswick coast revived following the mass transplants of resistant oysters from Prince Edward Island.

What seems to be happening in extensive culture is that partial steps toward disease control are taken in response to a crisis—enough to enable survival of the industry—but the measures are inadequate to prevent substantial economic losses, especially during epizootic periods. In intensive culture, control measures within a facility are more feasible, within the confines of cost effectiveness, whereas in extensive culture, environmental variables are more difficult to manipulate, and partial solutions, such as farming around a disease problem, must be used.

Disease control is complicated by the continuing interplay of host susceptibility, pathogen virulence, and environmental influences. Frank pathogens and their effects can be dealt with reasonably well, but *much of the damage to cultured populations is caused by facultative pathogens* (bacteria and viruses especially) which exert effects when water quality is not maintained or when other stressors (e.g., abnormal temperature, oxygen deficiency, inadequate diet, overcrowding) exist in culture facilities. Disease is often the overt symptom of marginal culture conditions; control frequently consists of improving those conditions.

V. CONCLUSIONS: EMERGING CONCEPTS IN SHELLFISH PATHOLOGY

Although it may seem a little unfair to other disciplines to isolate and emphasize disease control in shellfish aquaculture as a major technological objective, the reality is that *the health of cultured animals must be a primary management concern*. Once this mental hurdle is cleared and accepted as an operating principle, it becomes easy to construct a list of guidelines for disease management. Some of these are:

- Whenever animals are crowded together under abnormal conditions, an opportunity exists for the spread of infectious disease and for mortality of a significant part of the population.
- Disease in cultured populations assumes even greater significance than it does in natural populations for a number of reasons, including the following: (1) cultivated animals are often more crowded than those in the natural habitat, which facilitates the transmission of pathogens and parasites; (2) shellfish are often cultivated in areas where natural populations did not exist previously, and their absence under natural conditions suggests some environmental limiting factor(s); (3) some culture still depends largely on the capture and impoundment of juveniles that may be slightly injured during capture or already infected by certain pathogens; and (4) the nutrition of cultivated populations may be deficient.
- The maintenance of animal health is a continuing struggle to control known agents and to diagnose and control new pathogens as they appear.
- Water quality maintenance and stress reduction should be principal foci of facility and population management actions, since disease is often a consequence of inattention to either or both of these factors.
- Diseases caused by vibrios have already assumed great importance among cultivated marine fish and shellfish populations. In fact, the emergent role of halophilic vibrios is one of the most exciting present aspects of disease studies in marine aquaculture. The past few years have seen some elucidation of the identity of the pathogens involved, but the picture is still murky. The etiological agent(s) of ulcer disease in Japanese fish was recently described as having characteristics of *V. parahaemolyticus* (which causes human "summer sickness") and *V. anguillarum* (long known to cause red disease of eels). Krantz *et al.* (1969)

found *V. parahaemolyticus* in Chesapeake Bay blue crabs, and posulated that the *Vibrio* is a pathogen of crabs.

- Molluscan shellfish culture (at present based on the most advanced marine aquacultural practices) has repeatedly experienced mass mortalities from outbreaks of epizootic disease. Some control measures are available, but the extent of knowledge of the diseases involved is still so limited that effective control (particularly in natural waters) is not yet an actuality.
- Disease control is a continuous process. Protocols must be developed for the control of each disease; these protocols must from then on be a part of rearing methodology, and expertise must be available to cope with new disease problems as they appear.
- Although disease may affect cultivated shellfish at any life history stage, a number of recent reports suggest that larval and postlarval stages are particularly vulnerable. Systemic and fatal infections of larval Crustacea and Mollusca, due to a wide array of microorganisms, have been described.
- Disease control measures may vary significantly with life history stages, but are usually based on the triumvirate of diagnosis, prevention, and treatment.
- Disease control measures in extensive culture can be augmented by techniques of stock and environmental manipulation and by attention to exclusionary principles based on attempts to understand the consequences of transfers or introductions of species from other geographic areas. Transfers and introductions of species for aquaculture purposes can increase disease problems in cultivated populations. Susceptible animals may be introduced into areas in which particular diseases are enzootic or epizootic, or new diseases may be introduced with transfers from other geographic areas.
- Latent infections are important in maintaining disease (and some level of disease resistance) in natural populations. They provide a continuing source of infectious material and may become patent and cause epizootics as a consequence of environmental stress, such as that frequently found in aquacultural systems.
- Facultative pathogens, particularly but not exclusively of the *Vibrio*–*Pseudomonas*–*Aeromonas* group, may, under the stresses of low dissolved oxygen, high temperatures, dietary imbalances, or high population densities, occur in epizootic proportions in cultivated populations of shellfish. Mortalities caused by nonoptimum environments are thus attributed to disease. The species composition of microorganisms in the vicinity of aquaculture fac-

ities may shift drastically, due to eutrophication or shedding of pathogens from infected stocks. Disease outbreaks are clearly aspects of ecosystem dynamics.

- Effective disease control measures must consider environmental aspects as well as the direct host-pathogen relationship. Environmental factors include (but are not limited to) water quality, temperature, density of the population, and nutrition. Departure from the optimum for any single factor results in stress on the cultivated population. Stress can lead to the suppression of immune responses (resistance), increased susceptibility to facultative pathogens, and the development of latent infections into patency. Frequently, epizootics and mortalities in cultivated populations follow periods of abnormal stress from some form of acute aquaculture system failure or from the gradual development of nonoptimum environmental conditions.
- The use of chemoprophylaxis and chemotherapy is feasible in intensive culture systems, but not as a substitute for good facility and population management practices. Chemotherapy should be considered a last-resort method in disease control, if methods of prevention have failed, as was emphasized by Herman (1970) and by Snieszko (1974).

Disease has already proven to be, and will undoubtedly continue to be, a major problem in shellfish aquaculture. Technological progress in disease diagnosis and control has been rapid, so that our successes now begin to outnumber our failures. The process is never ending, though, as new disease entities and new crises appear, threatening aquaculture production.

REFERENCES

- Abrahams, D., and Brown, W. D. (1977). Toxicity to juvenile European lobster (*Homarus gammarus*) of several anti-fungal agents used to control *Haliphthoros milfordensis*. *Aquaculture* **12**, 31-40.
- Acton, R. T., Weinheimer, P. W., and Evans, E. E. (1969). A bactericidal system in the American lobster, *Homarus americanus*. *J. Invertebr. Pathol.* **13**, 463-464.
- Alderman, D. J. (1980). Shellfish disease problems—Past, present and future. *Proc. Shellf. Conf., Shellf. Assoc. G.B., 11th* pp. 30-40.
- Anderson, J. I. W., and Conroy, D. A. (1968). The significance of disease in preliminary attempts to raise Crustacea in sea water. *Proc. Symp. Mond. Comm. Off. Int. Epizoot. Étude Maladies Poissons, 3rd* **3**, 8 pp.
- Andrews, J. D. (1966). Oyster mortality studies in Virginia. V. Epizootiology of MSX, a protistan pathogen of oysters. *Ecology* **47**, 19-31.

- Andrews, J. D. (1968). Oyster mortality studies in Virginia. VII. Review of epizootiology and origin of *Minchinia nelsoni*. *Proc. Natl. Shellfish. Assoc.* **58**, 23–36.
- Andrews, J. D. (1976). Epizootiology of *Dermocystidium marinum* (= *Labyrinthomyxa marinum*) in oysters. *Proc. Int. Colloq. Invert. Pathol.*, 1st pp. 172–174.
- Andrews, J. D. (1979). Oyster diseases in Chesapeake Bay. *Mar. Fish. Rev.* **41**(12), 45–53.
- Andrews, J. D., and Frierman, M. (1974). Epizootiology of *Minchinia nelsoni* in susceptible wild oysters in Virginia, 1959 to 1971. *J. Invertebr. Pathol.* **24**, 127–140.
- Andrews, J. D., and Hewatt, W. G. (1957). Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. *Ecol. Monogr.* **27**, 1–26.
- Andrews, J. D., and Wood, J. L. (1967). Oyster mortality studies in Virginia. VI. History and distribution of *Minchinia nelsoni*, a pathogen of oysters, in Virginia. *Chesapeake Sci.* **8**, 1–13.
- Arie, B., and Stewart, J. E. (1984). Further developments in the induction of resistance in *Homarus americanus* to the bacterial disease gaffkemia. *Int. Counc. Explor. Sea, Doc. C.M.1984/F:9*, 3 pp.
- Armstrong, D. A., Buchanan, D. V., and Caldwell, R. S. (1976). A mycosis caused by *Lagenidium* sp. in laboratory reared larvae of the Dungeness crab, *Cancer magister*, and possible chemical treatments. *J. Invertebr. Pathol.* **28**, 329–336.
- Bang, F. (1975). Comparisons of immune mechanisms in vertebrates and invertebrates. In "Invertebrate Immunity" (K. Maramorosch and R. E. Shope, eds.), pp. 355–357. Academic Press, New York.
- Bayne, B. L. (1975). Aspects of physiological condition in *Mytilus edulis* L., with special reference to the effects of oxygen tensions and salinity. *Proc. Eur. Mar. Biol. Symp.*, 9th pp. 213–238.
- Bayne, C. J. (1983). Molluscan immunobiology. In "The Mollusca" (A. S. M. Saleuddin and K. M. Wilbur, eds.), Vol. 5, Part 2, pp. 407–486. Academic Press, New York.
- Bayne, B. L., and Scullard, C. (1977). Rates of nitrogen excretion by species of *Mytilus* (Bivalvia: Mollusca). *J. Mar. Biol. Assoc. U.K.* **57**, 355–369.
- Bayne, B. L., Moore, M. N., Widdows, J., Livingstone, D. R., and Salkeld, P. (1979). Measurement of the response of individuals to environmental stress and pollution: Studies with bivalve molluscs. *Philos. Trans. R. Soc. London, Ser. B* **286**, 563–581.
- Becker, C. D., and Pauley, G. B. (1968). An ovarian parasite (protista incertae sedis) from the Pacific oyster, *Crassostrea gigas*. *J. Invertebr. Pathol.* **12**, 425–437.
- Bland, C. E., and Amerson, H. V. (1973). Observations on *Lagenidium callinectes*: Isolation and sporangial development. *Mycologia* **65**, 310–320.
- Bland, C. E., Ruch, D. G., Salser, B. R., and Lightner, D. V. (1976). Chemical control of *Lagenidium*, a fungal pathogen of marine crustacea. *Univ. N.C. Sea Grant Program [Publ.] INC-SB-76-02*, 38 pp.
- Blogoslawski, W. J., Stewart, M. E., and Rhodes, E. W. (1978). Bacterial disinfection in shellfish hatchery disease control. *Proc. Annu. Meet.—World Maric. Soc.* **9**, 589–602.
- Boemare, N., Cousserans, F., and Bonami, J. -R. (1978). Epizootie à Vibrionaceae dans les élevages de crevettes Penaeidae. *Ann. Zool.—Ecol. Anim.* **10**, 227–238.
- Bowser, P. R., Rosemark, R., and Reiner, C. R. (1981). A preliminary report of vibrios in cultured American lobsters, *Homarus americanus*. *J. Invertebr. Pathol.* **37**, 80–85.
- Brown, C. (1973). The effects of some selected bacteria on embryos and larvae of the American oyster, *Crassostrea virginica*. *J. Invertebr. Pathol.* **21**, 215–223.
- Brown, C. (1981). A study of two shellfish-pathogenic *Vibrio* strains isolated from a Long Island hatchery during a recent outbreak of disease. *J. Shellfish Res.* **1**, 83–87.

- Brown, C., and Losee, E. (1978). Observations on natural and induced epizootics of vibriosis in *Crassostrea virginica* larvae. *J. Invertebr. Pathol.* **31**, 41–47.
- Brown, R. S. (1980). The value of the multidisciplinary approach to research on marine pollution effects as evidenced in a three year study to determine the etiology and pathogenesis of neoplasia in the soft-shell clam, *Mya arenaria*. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **179**, 125–128.
- Comps, M. (1970). Observations sur les causes d'une mortalité anormale des huîtres plates dans le Bassin de Marennes. *Rev. Trav. Inst. Pêches Marit.* **34**, 317–326.
- Comps, M., and Duthoit, J. L. (1976). Infection virale associée à la "maladie des branchies" de l'huître portugaise *Crassostrea angulata* Lmk. *C. R. Hebd. Séances Acad. Sci., Ser. D* **282**, 1595–1596.
- Comps, M., Bonami, J. R., Vago, C., and Campillo, A. (1976). Une virose de l'huître portugaise (*Crassostrea angulata* Lmk). *C. R. Hebd. Séances Acad. Sci., Ser. D* **282**, 1991–1993.
- Comps, M., Tige, G., and Grizel, H. (1980). Étude ultrastructurale d'une protiste parasite de l'huître *Ostrea edulis* L. *C. R. Hebd. Séances Acad. Sci., Ser. D* **290**, 383–384.
- Cook, D. W., and Lofton, S. R. (1973). Chitinoclastic bacteria associated with shell disease in *Penaeus* shrimp and the blue crab *Callinectes sapidus*. *J. Wildl. Dis.* **19**, 154–159.
- Corner, E. D. S., and Cowry, C. B. (1968). Biochemical studies on the production of marine zooplankton. *Biol. Rev.* **43**, 393–426.
- Couch, J. A. (1974). Free and occluded virus similar to *Baculovirus* in the hepatopancreas of pink shrimp. *Nature (London)* **247**, 229–231.
- Couch, J. A. (1978). Diseases, parasites, and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North America. *Fish. Bull.* **76**, 1–44.
- Couch, J. A., Farley, C. A., and Rosenfield, A. (1966). Sporulation of *Minchinia nelsoni* (Haplosporida, Haplosporidiidae) in the American oyster *Crassostrea virginica*. *Science* **153**, 1529–1531.
- Couch, J. A., Summers, M. D., and Courtney, L. (1975). Environmental significance of *Baculovirus* infections in estuarine and marine shrimp. *Ann. N.Y. Acad. Sci.* **266**, 528–536.
- Couch, J. N. (1942). A new fungus on crab eggs. *J. Elisha Mitchell Sci. Soc.* **58**, 158–164.
- D'Agostino, A. (1975). Antibiotics in cultures of invertebrates. In "Culture of Marine Invertebrate Animals" (W. L. Smith and M. H. Chanley, eds.), 338 pp. Plenum, New York.
- Davis, H. C., Loosanoff, V. L., Weston, W. H., and Martin, C. (1954). A fungus disease in clam and oyster larvae. *Science* **120**, 36–38.
- Egusa, S., and Ueda, T. (1972). A *Fusarium* sp. associated with black gill disease of the Kuruma prawn, *Penaeus japonicus* Bate. *Nippon Suisan Gakkaishi* **38**, 1253–1260.
- Elston, R. A. (1984). Prevention and management of infectious diseases in intensive mollusc husbandry. *J. World Maric. Soc.* **15**, 284–300.
- Elston, R., and Lockwood, G. S. (1983). Pathogenesis of vibriosis in cultured juvenile red abalone, *Haliotis rufescens* Swainson. *J. Fish Dis.* **6**, 111–128.
- Elston, R. A., and Wilkinson, M. T. (1985). Pathology, management and diagnosis of oyster velar virus disease (OVVD). *Aquaculture* **48**, 189–210.
- Elston, R., Elliott, E., and Colwell, R. R. (1982). Conchiolin infection and surface coating *Vibrio*: shell fragility, growth depression and mortalities in cultured oysters and clams, *Crassostrea virginica*, *Ostrea edulis* and *Mercenaria mercenaria*. *J. Fish Dis.* **5**, 265–284.
- Farley, C. A. (1967). A proposed life cycle of *Minchinia nelsoni* (Haplosporida, Haplosporidiidae) in the American oyster *Crassostrea virginica*. *J. Protozool.* **14**, 616–625.
- Farley, C. A., Banfield, W. G., Kasnic, G. K., and Foster, W. S. (1972). Oyster herpes-type virus. *Science* **178**, 759–760.

- Feng, S. Y., Khairallah, E. A., and Canzonier, W. J. (1970). Hemolymph free amino acids and related nitrogenous compounds of *Crassostrea virginica* infected with *Bucephalus* sp. and *Minchinia nelsoni*. *Comp. Biochem. Physiol.* **34**, 547–556.
- Fisher, W. S. (1976). Relationships of epibiotic fouling and mortalities of the eggs of the Dungeness crab (*Cancer magister*). *J. Fish. Res. Board Can.* **33**, 2849–2853.
- Fisher, W. S., and Nelson, R. T. (1977). Therapeutic treatment for epibiotic fouling on Dungeness crab larvae (*Cancer magister*) reared in the laboratory. *J. Fish Res. Board Can.* **34**, 432–436.
- Fisher, W. S., Nilson, E. H., and Shleser, R. A. (1975). Effect of the fungus *Haliphthoros milfordensis* on the juvenile stages of the American lobster *Homarus americanus*. *J. Invertebr. Pathol.* **26**, 41–45.
- Fisher, W. S., Rosemark, T. R., and Nilson, E. H. (1976a). The susceptibility of cultured American lobsters to a chitinolytic bacterium. *Proc. Annu. Meet.—World Maric. Soc.* **7**, 511–520.
- Fisher, W. S., Rosemark, T. R., and Shleser, R. A. (1976b). Toxicity of malachite green to cultured American lobster larvae. *Aquaculture* **8**, 151–156.
- Fisher, W. S., Nilson, E. H., Follett, L. F., and Shleser, R. A., (1976c). Hatching and rearing lobster larvae (*Homarus americanus*) in a disease situation. *Aquaculture* **7**, 75–80.
- Fisher, W. S., Nilson, E. H., Steenbergen, J. F., and Lightner, D. V. (1978). Microbial diseases of cultured lobsters: A review. *Aquaculture* **14**, 115–140.
- Ford, S. E., and Haskin, H. H. (1982). History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957–1980. *J. Invertebr. Pathol.* **40**, 118–141.
- Goggins, P. L., and Hurst, J. W., Jr. (1960). "Progress Report on Lobster Gaffkyemia (Red Tail)," unpublished mimeo rep. Dep. Sea Shore Fish., Augusta, Maine.
- Grizel, H., Comps, M., Bonami, J. -R., Cousserans, F., Duthoit, J. L., and Le Pennec, M. A. (1974). Recherche sur l'agent de la maladie de la gland digestive de *Ostrea edulis* Linné. *Sci. Pêche, Bull. Inst. Pêches Marit.* **240**, 7–29.
- Hada, H. S., West, P. A., Lee, J. V., Stemmler, J., and Colwell, R. R. (1984). *Vibrio tubiashii* sp. nov., a pathogen of bivalve molluscs. *Int. J. Syst. Bacteriol.* **34**, 1–4.
- Haskin, H. H., and Ford, S. E. (1979). Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. *Mar. Fish. Rev.* **41**(1–2), 54–63.
- Haskin, H. H., and Ford, S. E. (1982). *Haplosporidium nelsoni* (MSX) on Delaware Bay seed oyster beds: A host–parasite relationship along a salinity gradient. *J. Invertebr. Pathol.* **40**, 388–405.
- Haskin, H. H., and Ford, S. E. (1983). Quantitative effects of MSX disease (*Haplosporidium nelsoni*) on production of the New Jersey oyster beds in Delaware Bay, U.S.A. *Int. Counc. Explor. Sea, Doc. C.M.1983/Gen:7*, 21 pp.
- Haskin, H. H., Stauber, L. A., and Mackin, J. A. (1966). *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae): Causative agent of the Delaware Bay oyster epizootic. *Science* **153**, 1414–1416.
- Hatai, D., Nakajima, K., and Egusa, S. (1974). Effects of various fungicides on the black gill disease of the Kuruma prawn (*Penaeus japonicus*) caused by *Fusarium* sp. *Gyobyo Kenkyu* **8**, 156–160.
- Helm, M. M., and Smith, F. M., (1971). Observations on a bacterial disease in laboratory-cultured larvae of the European flat oyster *Ostrea edulis* L. *Int. Counc. Explor. Sea, Doc. C.M.1971/K:10*, 7 pp.
- Herman, R. L. (1970). Prevention and control of fish diseases in hatcheries. *Am. Fish. Soc., Spec. Publ.* **5**, 3–15.
- Iversen, E. S., and Kelly, J. F. (1976). Microsporidiosis successfully transmitted experimentally in pink shrimp. *J. Invertebr. Pathol.* **27**, 407–408.

- Jeffries, H. P. (1972). A stress syndrome in the hard clam *Mercenaria mercenaria*. *J. Invertebr. Pathol.* **20**, 242–251.
- Johnson, S. K. (1974a). Ectocommensals and parasites of shrimp from Texas rearing ponds. *Tex. A&M Univ. Sea Grant Program [Rep.] TAMU-SG-74-207*, 20 pp.
- Johnson, S. K. (1974b). *Fusarium* sp. in laboratory-held pink shrimp. *Tex. Agric. Ext. Serv., Fish Dis. Diagn. Lab., [Rep.] FDDL 1*, 2 pp.
- Johnson, S. K. (1976). Chemical control of peritrichous ciliates on young penaeid shrimp. *Tex. Agric. Ext. Serv., Fish Dis. Diagn. Lab. [Rep.] FDDL-S7*, 4 pp.
- Johnson, P. T. (1983). Diseases caused by viruses, rickettsiae, bacteria, and fungi. In "The Biology of Crustacea" (D. E. Bliss, ed.), Vol. 6, pp. 1–78. Academic Press, New York.
- Krantz, G. E., Colwell, R. R., and Lovelace, E. (1969). *Vibrio parahaemolyticus* from the blue crab *Callinectes sapidus* in Chesapeake Bay. *Science* **164**, 1286–1287.
- Leibovitz, L. (1978). Shellfish diseases. *Mar. Fish. Rev.* **40**, 61–64.
- Lewis, D. H. (1973). Response of brown shrimp to infection with *Vibrio* sp. *Proc. Annu. Meet.—World Maric. Soc.* **4**, 333–338.
- Lightner, D. V. (1975). Some potentially serious disease problems in the culture of penaeid shrimp in North America. *Proc. U.S.–Jpn. Nat. Resour. Program: Symp. Aquacult. Dis.* pp. 75–97.
- Lightner, D. V. (1977). Fungus (*Fusarium*) disease of shrimps. In "Disease Diagnosis and Control in North American Marine Aquaculture. Developments in Aquaculture and Fisheries Science" (C. J. Sindermann, ed.), Vol. 6, pp. 42–47. Elsevier, Amsterdam.
- Lightner, D. V., and Fontaine, C. T. (1973). A new fungus disease of the white shrimp *Penaeus setiferus*. *J. Invertebr. Pathol.* **22**, 94–97.
- Lightner, D. V., and Fontaine, C. T. (1975). A mycosis of the American lobster, *Homarus americanus*, caused by *Fusarium* sp. *J. Invertebr. Pathol.* **25**, 239–245.
- Lightner, D. V., and Lewis, D. H. (1975). A septicemic bacterial disease syndrome of penaeid shrimp. *Mar. Fish. Rev.* **37**(5–6), 25–28.
- Lightner, D. V., Redman, R. M., and Bell, T. A. (1983). Infectious hypodermal and hemato-poietic necrosis, a newly recognized virus disease of penaeid shrimp. *J. Invertebr. Pathol.* **42**, 62–70.
- Logie, R. R. (1956). Oyster mortalities, old and new, in the Maritimes. *Fish. Res. Board Can., Prog. Rep. Atl. Coast Stn.* **65**, 3–11.
- Mackin, J. G. (1962). Oyster disease caused by *Dermocystidium marinum* and other micro-organisms in Louisiana. *Publ. Inst. Mar. Sci. Univ. Texas* **7**, 132–229.
- Mackin, J. G., Owen, H. M., and Collier, A. (1950). Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* Gmelin. *Science* **111**, 328–329.
- Marteil, L. (1969). La maladie des branchies des huîtres portugaises des côtes françaises de l'Atlantique. Données générales sur la maladie des branchies. *Rev. Trav. Inst. Pêches Marit.* **33**(2), 145–150.
- Moore, M. N. (1976). Cytochemical demonstration of latency of lysosomal hydrolyses in digestive cells of the common mussel, *Mytilus edulis*, and changes induced by thermal stress. *Cell Tissue Res.* **175**, 279–287.
- Moore, M. N. (1977). Lysosomal responses to environmental chemicals in marine invertebrates. In "Pollutant Effects on Marine Organisms" (C. S. Giam, ed.), pp. 143–154. Heath, Lexington, Massachusetts.
- Nilson, E. H., Fisher, W. S., and Shleser, R. A. (1975). Filamentous infestations observed on eggs and larvae of cultured crustaceans. *Proc. Annu. Meet.—World Maric. Soc.* **6**, 367–375.

- Nilson, E. H., Fisher, W. S., and Shleser, R. A. (1976). A new mycosis of larval lobster (*Homarus americanus*). *J. Invertebr. Pathol.* **27**, 177–183.
- Oprandy, J. J., Chang, P. W., Pronovost, A. D., Cooper, K. R., Brown, R. S., and Yates, V. J. (1981). Isolation of a viral agent causing hematopoietic neoplasia in the soft-shell clam, *Mya arenaria*. *J. Invertebr. Pathol.* **38**, 45–51.
- Paterson, W. D., and Stewart, J. E. (1974). In vitro phagocytosis by hemocytes of the American lobster (*Homarus americanus*). *J. Fish. Res. Board Can.* **31**, 1051–1056.
- Pichot, Y., Comps, M., Tige, G., Grizel, H., and Rabouin, M. (1980). Recherches sur *Bonamia ostreae* gen. n., sp. n., parasite nouveau de l'huître plate *Ostrea edulis* L. *Rev. Trav. Inst. Pêches Marit.* **43**, 131–140.
- Pichot, Y., Comps, M., and Deltreil, J. P. (1981). Recherches sur *Haplosporidium* sp. (Haplosporida–Haplosporidiidae) parasite de l'huître plate *Ostrea edulis* L. *Rev. Trav. Inst. Pêches Marit.* **43**, 405–408.
- Ray, S. M. (1954). Biological studies of *Dermocystidium marinum*, a fungus parasite of oysters. *Rice Inst. Pam., Spec. Issue* pp. 1–114.
- Ray, S. M., and Chandler, A. C. (1955). *Dermocystidium marinum*, a parasite of oysters. *Exp. Parasitol.* **4**, 172–200.
- Sano, T., Nishimura, T., Oquuma, K., Momoyama, K., and Takeno, N. (1981). Baculovirus infection of cultured Kuruma shrimp, *Penaeus japonicus* in Japan. *Gyobyo Kenkyu* **15**, 185–191.
- Schapiro, H. C., Mathewson, J. H., Steenbergen, J. F., Kellogg, S., Ingram, C., Nirengarten, G., and Rabin, H. (1974). Gaffkemia in the California spiny lobster, *Panulirus interruptus*: Infection and immunization. *Aquaculture* **3**, 403–408.
- Schapiro, H. C., and Steenbergen, J. F. (1974). Active immunity to gaffkemia in lobsters. *Proc. Annu. Meet.—World Maric. Soc.* **5**, 195–197.
- Selye, H. (1950). Stress and the general adaptation syndrome. *Br. Med. J.* **1**, 1383–1395.
- Selye, H. (1955). Stress and disease. *Science* **122**, 625–631.
- Selye, H. (1976). "The Stress of Life," 515 pp. McGraw-Hill, New York.
- Sindermann, C. J. (1970a). "Principal Diseases of Marine Fish and Shellfish," 369 pp. Academic Press, New York.
- Sindermann, C. J. (1970b). Disease and parasite problems in marine aquaculture. In "Marine Aquaculture" (W. J. McNeil, ed.), pp. 103–134. Oregon State University Press, Corvallis, Oregon.
- Sindermann, C. J. (1979). Oyster mortalities and their control. In "Advances in Aquaculture" (T. V. R. Pillay and W. A. Dill, eds.), pp. 349–361. Fish. News Books, Farnham, Surrey, England.
- Sindermann, C. J., and Lightner, D. V. (Eds.) (1988). "Disease Diagnosis and Control in North American Marine Aquaculture," 2nd ed., 431 pp. Elsevier, Amsterdam.
- Smith, A. C. (1983). Simple test for general stress in a decapod crustacean, the pink shrimp *Penaeus duorarum*. *J. Aquaricult. Aquat. Sci.* **3**, 11–12.
- Snieszko, S. F. (1974). The effects of environmental stress on outbreaks of infectious diseases of fishes. *J. Fish Biol.* **6**, 197–208.
- Stewart, J. E., and Arie, B. (1974). Effectiveness of vancomycin against gaffkemia, the bacterial disease of lobsters (genus *Homarus*). *J. Fish. Res. Board Can.* **31**, 1873–1879.
- Telford, M. (1974). Blood glucose in crayfish. II. Variations induced by artificial stress. *Comp. Biochem. Physiol. A* **48**, 555–560.
- Tharp, T. P., and Bland, C. E. (1977). Biology and host range of *Haliphthoros milfordensis* Vishniac. *Can. J. Bot.* **55**, 2936–2944.
- Tubiash, H. S. (1975). Bacterial pathogens associated with cultured bivalve mollusk larvae.

- In "Culture of Marine Invertebrate Animals" (W. L. Smith and M. H. Chanley, eds.), pp. 61-71. Plenum, New York.
- Tubiash, H. S., Chanley, P. E., and Leifson, E. (1965). Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. I. Etiology and epizootiology. *J. Bacteriol.* **90**, 1036-1044.
- Vago, C. (1966). A virus disease in Crustacea. *Nature (London)* **209**, 1290.
- Vanderzant, C., Nickelson, R., and Parker, J. C. (1970). Isolation of *Vibrio parahemolyticus* from Gulf Coast shrimp. *J. Milk Food Technol.* **33**, 161-162.
- Villella, J. B., Iversen, E. S., and Sindermann, C. J. (1970). Comparison of the parasites of pond-reared and wild pink shrimp. (*Penaeus duorarum* Burkenroad) in South Florida. *Trans. Am. Fish. Soc.* **99**, 789-794.
- Vishniac, H. S. (1955). The morphology and nutrition of a new species of *Sirolopidium*. *Mycologia* **47**, 633-645.
- Vivares, C. P., Bonami, J. -R., and Jaspers, E. (eds.) (1986). Pathologie en Aquaculture Marine. *Eur. Aquacult. Soc., Spec. Publ.* **9**, 428 pp.
- Wood, J. L., and Andrews, J. P. (1962). *Haplosporidium costale* (Sporozoa) associated with disease of Virginia oysters. *Science* **136**, 710-711.

Part VI

Disease as a Causal Factor in Mass Mortalities of Shellfish

Many factors may affect the abundance of molluscan and crustacean shellfish. Prominent among them are man-made environmental changes, such as dredging, toxic chemicals, and abnormal nutrient loads, leading to algal blooms and anoxia. Other environmental changes, such as extreme temperatures or salinities, changes in predator abundance, storms, inadequate food production, or disease outbreaks, may also influence survival. Superimposed on these environmental influences is, of course, the effect of fishing (and frequently of overfishing) on stocks.

Except for that part of total mortality which results from fishing, it is often difficult to relate observed mortalities to specific environmental events—except in instances of catastrophic occurrences, such as widespread anoxia, severe storms, or disease epizootics. However, during the past several decades, as diagnostic methods have improved, outbreaks of specific pathogens have been associated with many mortalities, and a reasonably clear cause-and-effect relationship has been established. What is emerging is a significant amount of information demonstrating that epizootic disease *is* a major factor in mortalities of shellfish.

Effects of epizootic diseases on shellfish populations, principally in the form of mortalities, have been observed and reported in the scientific literature since early in the 20th century, and a substantial body of literature exists—especially for bivalve molluscs such as oysters. Effects on shellfish are easier to assess than are those on fish populations, primarily because of their limited mobility and because (in the case of most molluscs) of the persistence of shells as records of mortalities. This greater amenability to the quantitative assessment of disease effects makes a consideration of the effects of shellfish diseases particularly critical.

With attention focused on epizootics, it is easy to overlook the sub-

lethal background disease effects on shellfish. These include a reduction in fecundity, debilitation, poor condition, slow growth, abnormal coloration, and behavioral modifications. Chronic diseases which cause these conditions may be progressive and directly lethal with time or they may act indirectly, by rendering the affected individuals more vulnerable to predators or other environmental stressors.

13

Epizootics and Mass Mortalities of Shellfish

I. INTRODUCTION

Many physical, chemical, and biological variables may contribute directly or indirectly to mortalities of commercial marine shellfish (Fig. 50). The actual cause of death in many mass mortalities is often undetermined, even after exhaustive studies. Disease has been suspected as a cause, but the actual disease agent has often proven to be elusive.

Less spectacular mortalities, which may also have severe continuing depressive effects on host population size, are probably more common than large-scale, or mass, mortalities. Minor fluctuations in abundance may be attributable to such "background" mortalities. Also, parasites and diseases that do not kill the host directly may act as indirect agents of mortality. Abnormal individuals are rendered more vulnerable to predation in many ways: Their body muscles may be partially destroyed, occlusion or erosion of their gills may interfere with respiration, or their normal behavior or protective coloration may be modified or obscured. For example, Hopkins (1957) observed that blue crabs prey more frequently on oysters which cannot close their shells as quickly or as tightly as normal oysters. Any increase in parasite burden must reduce the probability of survival in an environment in which death, early and sudden, is the rule rather than the exception. For parasites with complex life cycles involving two or more hosts, consumption of an earlier host in the cycle—one weakened by the parasite—by the right predator may be critical to the completion of the cycle.

Another prominent effect of parasitization of marine molluscs and crustaceans is sterilization of the host. Larval trematodes are notable for destroying the gonads of gastropods and bivalves, and parasitic barnacles and epicaridean isopods produce similar effects in crustaceans. In areas where levels of parasitization are high, the reproductive capacity



Figure 50. Surf clams (*Spisula solidissima*) washed ashore on a New Jersey beach after a severe winter storm.

of the host population may be seriously impaired. In a study of the ecological relationship of the marine snail, *Littorina littorea*, and its trematode parasite, *Cryptocotyle lingua*, Sindermann and Farrin (1962) and Sindermann (1965) found prevalences of the parasite of over 50% in certain coastal areas, indicating that the reproductive potential of those snail populations was suppressed by that amount. An excellent review of this counterpart "parasitic castration" of Crustacea has been provided by Reinhard (1956).

The effects of disease can be categorized generally as catastrophic, resulting in mass mortalities, or continuing, producing a constant drain on population numbers. Although disease is always present, and mortalities have occurred in the past, new factors have been introduced by humans to set the stage for the spread of epizootic disease. For example, oysters are transferred promiscuously from one geographic area to another; populations are often crowded in dense beds, sometimes in areas where natural populations did not exist previously; drastic physical and

chemical changes have been made in oyster habitats; and new predators have been introduced. A dominant mortality factor—disease—has been aided by human activities; it must be controlled if we are to achieve maximum production of cultivated inshore molluscs and crustaceans.

It is, of course, true that many different environmental factors—physical, chemical, and biological—can kill oysters, crabs, or other animals of commercial value (Brongersma-Sanders, 1957). Any single factor may become overriding, however, at a particular time in the life of a species, and in this chapter several examples are given of how the factor of disease can reduce the abundance of marine species. Furthermore, it is very likely that many mass mortalities are the consequences of interaction of several environmental and population factors.

It is easy, of course, to overextend any point of view; there is no implication here that every decrease in abundance can be blamed on disease. An excellent case could also be made from the published literature for the significant role of predation, particularly during population peaks of particular predator species, as a major cause of fluctuations in abundance of commercially valuable species. Man-made changes in the environment can also affect abundance. Because of industrial pollution, some shellfish populations have been eliminated from certain localized areas within estuaries and along the coast. In addition, other types of pollution have made extensive areas in rivers and bays unavailable for the harvesting of shellfish. It is likely that mass mortalities are, and have always been, natural methods of population regulation, but, until recently, these mortalities would have been accepted with the same dazed bewilderment and inaction that must have characterized the behavior of our ancestors during the plagues of the Dark Ages. We can now look to methods of environmental control and stock manipulation, particularly for sedentary shallow-water species, such as oysters, clams, and even certain Crustacea, as part of the methodology of an increasingly complex system of cultivation of inshore waters.

This chapter reviews some of the information on disease-caused mass mortalities of molluscan and crustacean shellfish. It represents a somewhat artificial but probably useful isolation of one environmental factor—epizootic disease—critical to the survival of aquatic populations.

II. EPIZOOTICS AND MASS MORTALITIES OF MOLLUSCS

Most of the great fisheries of the world have experienced major fluctuations in supply. Causes of such fluctuations have been much discussed, but rarely determined precisely. Among the factors implicated in reduc-

tions in abundance are overfishing, failure of spawning, inadequate food supply, disease, sudden and drastic changes in the environment, and a host of other factors. Among marine populations such as oysters, which occur in shallow coastal waters, mass mortalities have occurred repeatedly in this century and have resulted in significant reductions in production—sometimes with long-term effects. Identification of precise causes has proven to be very difficult; only a few mass mortalities have been definitely associated with specific pathogens, predators, or other environmental factors.

It might be good at this point to discuss a few generalizations about mortalities and their causes—stating at the outset that mass mortalities are merely an exaggerated form of natural mortality, usually distinguished by rapid onset, and affecting a large proportion of the population over an extensive geographic area. Many mass mortalities of molluscs undoubtedly result from interactions of environmental and biological factors. Only a few have been clearly associated with a single, overriding causal factor, and of these few, disease has usually been the identifiable cause. "Disease," in its broadest sense, can be defined as "any departure from the normal structure or function of a living organism." Origins may be infectious or noninfectious. Dysfunction and death due to the activity of infectious agents constitute the narrower, but often predominant, concept of disease. Infectious diseases—caused by viruses, bacteria, fungi, protozoa, and other parasites—are usually prime suspects in the searches for causes of mortalities, sometimes to the exclusion of other possible causes. In many instances of mass mortalities, it is probably the combination of an infectious agent and environmental stress that eventually kills animals on a large scale.

This section on mortalities of molluscs emphasizes oysters—logically, since this is where the greatest concentration of literature exists. Such an emphasis should not obscure the fact that mortalities have been observed and reported in other species—especially mussels, scallops, and clams. Before looking in depth at oyster mortalities, a few examples from other molluscan groups should be appropriate.

Mussel mortalities have been reported sporadically throughout most of this century. Hirsch (1921), Dollfus (1923), and Korringa (1952) reported major mortalities of *Mytilus edulis* in Europe, probably due to a contagious disease, in 1900–1919. The disease reached a peak in 1914–1916. Sick mussels of all ages lost their byssal attachment, mantles were retracted, meats were thin, and adductor muscles were weak. Histological and bacteriological examinations were inconclusive. More recently (1977), mortalities of mussels were reported from Prince Edward

Island, Canada. Dying animals had generalized signs of disease—such as emaciation and weak adductor muscles—and a *Perkinsus*-like protozoan parasite was seen in the tissues (Li and Clyburne, 1979).

Evidence for mass mortalities in deep-water molluscs has been published. Merrill and Posgay (1964), estimating natural mortality rates of sea scallops, *Placopecten magellanicus*, from Georges Bank by ratios of dead shells (clappers) to live animals, found ratios as high as 0.83, suggesting extensive mortalities. Medcof and Bourne (1964), also working with sea scallops of the western North Atlantic Ocean, identified a number of causes of natural mortality, including parasites and shell pests. They found no evidence of mortality from pathogenic microorganisms, although the flagellate *Hexamita* was isolated from scallops. Dickie and Medcof (1963) reported nine mass mortalities of scallops (up to 80% of existing populations) in the southern Gulf of St. Lawrence since 1928, which may have limited the abundance of fishable stocks. These authors concluded that sudden increases in water temperature were directly responsible. Other less spectacular mortalities (up to 25%) were believed to have been caused by unusual concentrations of predators. Evidence suggestive of mass mortalities of Australian scallops has been supplied by Sanders (1966). Explorations for new beds off the coast of Victoria disclosed extensive concentrations of dead shells, but no living scallops in an area which had supported a fishery a decade earlier. A more localized mass mortality, thought to be associated with a rickettsial organism, occurred in sea scallops, *P. magellanicus*, in Narragansett Bay, Rhode Island, in 1979–1980. Three quarters of the population died in a 3-month period, and the remainder died in the following 6 months. Deaths were attributed to degenerative changes caused by gill and muscle infections (Gulka *et al.*, 1983).

Soft-shell clam, *Mya arenaria*, populations of the Northumberland Strait region of the Gulf of St. Lawrence were found by Ingalls and Needler (1939, 1940) to have suffered mass mortalities in those years. Evidence of recent deaths, in the form of decomposing meats, was recorded for many beds, and quahogs, *Mercenaria mercenaria*, were also found to have suffered heavy recent mortality. Not all bays were affected, and the authors gave no indication of the possible causes of the mortalities observed.

Other sporadic accounts of mortalities of bivalve molluscs of commercial importance describe severe effects of a fatal hemocytic sarcoma in soft-shell clams, *M. arenaria*, from Chesapeake Bay. An epizootic occurred in 1983–1985, with peak prevalences of 42–65%. Concurrent laboratory studies indicated 100% lethality of infections, and some local

clam-producing beds were severely affected. Production of clams from the entire area declined by 50% from 1983 to 1985 (Farley *et al.*, 1986; Farley, 1989).

But these are only preliminaries to the extensive information on mortalities of oysters—on a worldwide basis—which is reviewed in the following section.

A. Epizootics and Mass Mortalities of Oysters

Major oyster mortalities are a matter of record in most of the world's principal production areas, where they have often caused significant population reductions. Some of these major mortalities have been ascribed to epizootics caused by infectious agents; many, however, remain of unknown origin, despite intensive scientific investigation. European, Japanese, and North American oyster populations have been affected in this century by catastrophic mass mortalities, in some instances bringing the local oyster industry to a standstill for a number of years.

In addition to the widespread catastrophic mortalities, many other mass deaths of a more localized nature occur in oyster populations, and there are also continuing background mortalities, which may at times reach levels of concern to the shellfish industry. A reduction of the impact of mortalities on production usually takes the form of adapting culture practices to take advantage of salinity, temperature, or other limitations on the activities of the causative factor(s).

In this section the world experience with mass mortalities of oysters in the 20th century is reviewed briefly, with particular attention to causes, effects, and control measures, if any. Oyster mortalities have been and are a matter of great concern to a number of nations which harvest wild stocks or, more frequently, cultivate oysters in coastal and estuarine waters. Within the past two decades alone mass mortalities have affected production of oysters in Japan, France, the United States, the United Kingdom, and The Netherlands. Earlier mortalities also affected production in these and other countries as well. Mortalities in some producing areas have exceeded 95% of the existing populations in a single growing season; in other areas mortalities have been lower, but often repeated for a number of years before abating. Effects, in terms of lost production, can be and have been determined accurately.

Although mass mortalities of oysters are natural phenomena, humans have done several things to increase the likelihood of their occurrence. We have crowded individuals into dense populations, often under marginal environmental conditions; we have transferred stocks freely, leading to introductions of predators and diseases not previously known

in the receiving area (Alderman, 1974); and we have modified the estuarine oyster production areas by dredging, dam construction, the use of fertilizers, and the release of chemical contaminants.

In considering the information about mass mortalities of oysters, we should not overlook the continuing background mortalities that are less spectacular, but not less significant, in determining population size. These mortalities are represented by the slow, continuous extraction of individuals from the population by deaths due to inadequate food, salinity extremes, predation, temperature extremes, siltation, low oxygen levels, crowding effects, etc. Actually, the dividing line between background mortality and mass mortality is hard to define and is only a matter of degree. As a very rough criterion, maximum background mortality in many oyster populations can be considered to be 10–15% in a single year (exclusive of larval and spat mortality); anything above that figure should probably be termed a mass mortality. This estimate is, of course, variable from area to area; some oyster growers become alarmed if annual mortalities exceed 5%, while others in different geographic areas tolerate annual mortalities approaching 50%. Other definitions of mass mortality exist. Koganezawa (1975) defined it as the death annually of 50–60% of the oysters, which he considered the boundary of existence for Japanese commercial oyster culture.

It should also be recognized that localized mass mortalities, involving relatively restricted geographic areas, can and do occur and can produce significant localized effects. Predators such as starfish, crabs, polyclad flatworms, or oyster drills can increase in abundance explosively and kill large numbers of oysters—particularly spat and seed. Changes in the physical environment, such as severe flooding of rivers, with a consequent reduction of salinities over oyster beds near river mouths, can cause localized, often complete, mortalities (Nikolić, 1964). Such local mass mortalities undoubtedly occur by the hundreds, as compared to one of the major widespread mortalities discussed in this chapter.

With the qualifications mentioned above, it seems that natural mortality of oysters can be considered (somewhat artificially, perhaps) in three aspects: (1) background mortality, a continuous, often unnoticed, but very significant reduction in numbers of individuals at any stage of the life history; (2) localized mass mortalities, affecting restricted geographic areas and killing up to 100% of the populations in those areas; and (3) widespread and extensive mass mortalities, affecting large geographic areas and reducing oyster production in these areas significantly. This chapter, confined largely to the third type of mortality, is an attempt to distill the often frustrating research—over a time span of

almost a century—designed to understand the nature and the causes of catastrophic mortalities and to find control measures.

Some earlier efforts have been made to summarize information about oyster mortalities (Gross and Smyth, 1946; Mackin, 1961; Sindermann, 1968a,b, 1970, 1976; Sprague, 1971). Because of the worldwide economic importance of oysters and because they have long been cultivated in shallow estuarine and coastal waters, it is only natural that mortalities, when they occur, would be observed and examined. Mortalities will be considered chronologically, by major geographic areas. Table 18 lists those to be considered.

1. Oyster Mortalities in Europe

The history of oyster mortalities in Europe is long and at times confusing. Species of consequence there are the flat oyster, *Ostrea edulis*, and the Portuguese oyster, *Crassostrea angulata*. Recently, the Pacific oyster, *Crassostrea gigas*, has been introduced on a large scale on the French coast.

Among the earliest mass mortalities of oysters reported in the scientific literature was one which occurred in the Arcachon basin, France, in 1877 (Hornell, 1910; Dollfus, 1921; Orton, 1937). The cause was not determined, but characteristics of dying oysters led to the application of the name "*maladie du pied*" to the disease condition. The disease was localized in the shell under the attachment of the adductor muscle, where it caused roughening and blistering of the shell and degeneration of adjacent muscle tissue. The muscles often became detached as irregular cysts were formed. Giard (1894) described a possible etiological agent as a bacterium, *Myotomus ostrearum*, but subsequent studies have cast doubt on this conclusion. It seems now that *maladie du pied* was a fungus-induced shell disease, which is considered later.

The next major European oyster mortalities in chronological sequence began in 1919. Catastrophic mortalities of *O. edulis* occurred in western Europe. Deaths began in 1919 in Mar Piccolo, near Taranto, Italy (Cerruti, 1941), and soon after were reported in England and in other European countries, where they persisted until 1923. Orton (1924a,b), who carried out exhaustive and painstaking investigations during this period, suspected but was unable to demonstrate a bacterial pathogen. Although no infectious agent could be directly associated with the mortalities, disease signs such as mantle retraction, pale digestive glands, muscle degeneration, gut inflammation, and shell and mantle pustules were seen. Ulcerations and pustules on the body and the mantle and shell pustules containing dead or moribund hemocytes were observed

Table 18. Major Oyster Mortalities during the Past Century

Year(s) of Mortality	Species Affected	Affected Area	Probable Cause
1877– ^a	<i>Ostrea edulis</i>	France	<i>Maladie du pied</i> (probably the fungus <i>Ostracoblabe implexa</i>)
1915– ^a	<i>Crassostrea gigas</i>	Japan (Kanasawa Bay)	Unknown
1915–1957	<i>C. virginica</i>	Canada (Gulf of St. Lawrence)	Malpeque Bay disease, cause unknown
1919–1923	<i>O. edulis</i>	Europe	Unknown
1924– ^a	(<i>Saccostrea commercialis</i>)	Australia (New South Wales)	Unknown
1927–1937	<i>C. gigas</i>	Japan (Miura Peninsula)	Unknown
1930– ^a	<i>O. edulis</i> , <i>Crassostrea angulata</i>	Europe	Shell disease, caused by the fungus <i>O. implexa</i>
1945–1955	<i>C. gigas</i>	Japan (Hiroshima Bay)	Unknown (bacterial infection suggested)
1949–present	<i>C. virginica</i>	United States (Gulf of Mexico)	Protozoan <i>Perkinsus marinus</i>
1957–present	<i>C. virginica</i>	United States (Atlantic coast)	Haplosporidians <i>Haplosporidium nelsoni</i> , <i>Haplosporidium costale</i>
1961– ^a	<i>C. gigas</i>	Japan (Matsushima Bay)	Unknown (physiological and environmental factors may be involved)
1963–1969	<i>C. gigas</i>	United States (Pacific coast)	Unknown (possible bacterial etiology)
1967–1970, 1970–1977	<i>C. angulata</i> and (later) <i>C. gigas</i>	France	Gill disease, caused by two iridoviruses
1968–1979	<i>O. edulis</i> (and to a lesser extent) <i>C. angulata</i>	Europe	Digestive gland disease, caused by the ascetosporan <i>Marteilia refringens</i>
1968–1978	<i>S. commercialis</i>	Australia (Queensland)	Ascetosporan <i>Marteilia sydneyi</i>
1979–present	<i>O. edulis</i>	Europe	Ascetosporan <i>Bonamia ostreae</i>

^a The exact year when mortalities ceased is not adequately reported in the literature available. In some instances mortalities did not cease, but merely receded into background levels.

in oysters from England and from The Netherlands during the mortality (Orton, 1937). These signs are quite likely to be associated with an infectious disease process. In addition to the exhaustive studies of Orton, Eyre (1923, 1924) isolated a fungus, *Cladothrix dichotoma*, and nine species of bacteria from sick and healthy oysters, but expressed doubt that any were true pathogens.

Korringa (1952) has given an excellent historical account of these mortalities of 1919–1923. Cultured oyster beds in France, England, Denmark, Germany, and The Netherlands were affected almost simultaneously, beginning in late 1919, peaking in 1920, and abating in 1921–1923. In Ireland the peak was in 1921. Many natural beds were also destroyed. A few isolated populations (Helgoland, Germany, and Brittany, France) were not affected until several years later. Mortalities did not occur in *C. angulata*. Although environmental factors such as poor food supply and low temperatures were claimed to be the causes of the catastrophic mortalities by a few authors (Gaarder and Alvsaker, 1951; Spärck, 1950), the available evidence strongly indicates an infectious disease (Cole, 1951; Fischer, 1951; Korringa, 1952).

Beginning in 1930 new mortalities affected *O. edulis* stocks in The Netherlands. Deaths were attributed to shell disease (Korringa, 1947, 1951a,b), thought to be caused by a fungus. The disease condition had been known in The Netherlands since 1902, but began an explosive increase in the Oosterschelde estuary in 1930, following implementation of a program for the widespread use of cockleshells as spat collectors.

The disease was characterized by the formation of green or brown pustules on the inner shell surfaces. Activity of the fungus varied directly with temperature. Thin parts of oyster shells were perforated by the disease agent, which proliferated after reaching the interior surfaces. The fungus was reported as a species of *Monilia* by Voisin (1931), who found the infection in 40% of the oysters imported into France from The Netherlands in 1931. Cole (1950) and Cole and Waugh (1956) reported infections in *O. edulis* from Brittany, and in *C. angulata* grown in England. Infections were common in beds where old shells were abundant. Cole and Hancock (1956) found the disease in almost all beds of native European oysters in England and described two distinct forms: the typical one, characterized by greenish, rubbery warts and knobs on the inside of the shell, particularly in the region of the adductor muscle attachment; and an atypical form, in which young oysters had thickened shells with numerous white patches but no deformation of the muscle attachment area. The shells of affected oysters were distorted, the condition index was reduced, and the adductor muscle often became detached.

A subsequent severe outbreak of shell disease with high mortalities

occurred in the summer of 1947, and the disease is now known throughout western Europe in *O. edulis* and *C. angulata*. Work by Alderman and Jones (1967, 1971) identified the causative organism as the phycomycete *Ostracoblabe implexa*, and those authors considered shell disease and *maladie du pied* (discussed earlier) to be identical.

Beginning in 1967, new oyster mortalities began to affect production in western Europe. Some of the mortalities have been attributed to gill disease, which was first reported by Marteil (1968, 1969) and by Comps (1969) in *C. angulata* from the French coast. It was almost simultaneously reported in Portugal (Vilela, 1968) and in the United Kingdom (Howell *et al.*, 1968). Alderman (1969) and Alderman and Gras (1969) reported severe mortalities (in some instances, over 90%) of *C. angulata* imported to England from Portugal in 1969. Mortalities were also severe in 1968 and in 1969 in Portuguese beds (Vilela and Sampayo, 1969). Gross indications of abnormality were similar to those seen in 1967 and in 1968 in France, where mortalities were also severe (reaching 80% in the Marennes–Oleron region). Affected oysters had thin, yellowish meats, with damage to the gills and the palps, and to a lesser extent the adductor muscle and the mantle, in the form of yellow–green pustules which increased in size and developed into extensive brownish areas of necrosis. The disease condition was described in both active and remission stages (the remission stage apparently lacking the yellow staining and brownish necrotic areas).

The precise etiology of gill disease remained in doubt for some time, until an iridovirus was associated with the 1967–1970 outbreak (Comps and Duthoit, 1976). An interesting aspect of the *C. angulata* gill disease is that a second outbreak, apparently caused by a different viral agent (but also an iridovirus), began in 1970, resulting in new mortalities and further reducing the surviving stocks (Comps *et al.*, 1976; Marteil, 1976; Bonami, 1977; Comps, 1978). Mortalities continued at high levels through 1973 in the Arcachon and Gironde production areas of France, reducing stocks drastically. Only the replacement of *C. angulata* by *C. gigas* in the Arcachon basin enabled continued production there, according to Deltreil (1973). A method to reduce the effects of gill disease was proposed by Deltreil (1969a,b, 1971). Oysters not sold but remaining on beds after 3 years were heavily infected and could constitute a reservoir of infection. Deltreil suggested the destruction of all oysters unsold at 4 years, and preferably at 3 years.

Another disease, called aber disease, or preferably digestive gland disease, was reported as a cause of mortalities in the flat oyster, *O. edulis*, on the French (Brittany) coast at about the same time (Comps, 1970; Herrbach, 1971; Bonami *et al.*, 1971). Mortalities began in 1967, con-

tinued to spread until 1972, and began regressing in 1973 (Grizel and Tige, 1973; Tige and Morel, 1974). Characteristic disease signs included poor condition index, yellowing of the digestive gland, glycogen loss, and tissue necrosis. One histological feature of significance was the presence in the digestive gland tubule epithelium (and occasionally in the gills and the palps) of a protozoan unlike any oyster pathogen whose life cycle had been described. A subsequent paper by Grizel *et al.* (1974a,b) named the parasite *Marteilia refringens*, without establishing its taxonomic position. Perkins (1976) used ultrastructural studies to demonstrate affinities of the organism to the protozoan phylum Ascomycota. Rosenfield (1976) also concluded independently (based on examination of the photomicrographs by Bonami *et al.*, 1971) that the parasite observed in the digestive gland disease of *O. edulis* was probably a haplosporidan (ascetosporan).

The digestive gland disease was considered by French workers to be specific for *O. edulis*, but a similar histopathology was seen in *C. angulata* populations undergoing massive mortalities on the southern coast of Spain (Gutiérrez and Pascual, 1975). Mortalities of up to 90% occurred in certain growing areas in 1973 and in 1974.

Then, in 1979 still another disease, bonamiasis, caused by the protozoan *Bonamia ostreae*, began affecting *O. edulis* populations in Britain—soon spreading to other French oyster-producing areas and then appearing in The Netherlands, Spain, Denmark, the United Kingdom, and the United States (Comps *et al.*, 1980; Balouet *et al.*, 1983; Elston *et al.*, 1986). The disease caused extensive gill lesions and the destruction of hemocytes; infections characteristically disclosed the presence of small (1- to 3- μ m) spherical parasites, called microcells. Infections were seen year-round, and mortalities due to bonamiasis in some French growing areas reached 80% (Poder *et al.*, 1982).

Although difficult to sort out specifically, it seems that during the past two decades in Europe (especially in France) three, possibly four, disease conditions have affected oysters and caused severe mortalities: gill disease, principally in *C. angulata* and to a lesser extent in *O. edulis* and *C. gigas*, caused by one and possibly two iridoviruses; digestive gland disease of *O. edulis*, caused by the ascetosporan *Marteilia refringens*; and bonamiasis, also in *O. edulis*, caused by the ascetosporan *Bonamia ostreae*.

Examining this impressive array of European oyster mortalities during the past century, and seeing the evidence (however inconclusive) that activities of infectious agents were probably involved in most of them, it is easy to conclude, as Mackin (1961) did earlier, that "of all

causes of mortality, disease ranks first," and that "all oyster-producing bays are endemic areas for one or more diseases."

2. *Oyster Mortalities in Asia*

The Japanese literature contains numerous historical accounts of mass oyster mortalities of unknown etiology dating back to 1915. Although disease was sometimes suspected, specific pathogens were usually not identified. Takeuchi *et al.* (1960) mentioned large-scale deaths of oysters in Kanasawa Bay, beginning in 1915 and continuing for a number of years. Over 80% of the oysters in that bay died annually. Ogasawara *et al.* (1962) reported similar mass mortalities on the Miura peninsula, beginning in 1927 and continuing for 10 years. Oyster farms all along the coast of the peninsula lost 50–80% of their crop annually.

More recently, large-scale mortalities of 2-year-old Pacific oysters, *C. gigas*, occurred in Hiroshima Bay and adjacent localities, beginning in 1945 (Fujita *et al.*, 1953, 1955). A 10-year study (Takeuchi, 1963; Takeuchi *et al.*, 1955, 1956, 1957, 1960) provided somewhat inconclusive evidence that a bacterial pathogen was responsible for the mortalities. Takeuchi *et al.* (1960) implicated a gram-negative, motile, 1- to 3- μ m bacillus, probably an *Achromobacter*, although the evidence presented was incomplete. Experimental infections were achieved with cultured bacteria, but the organisms could be isolated from healthy as well as sick oysters, and also from seawater. Moribund oysters were characterized by diffuse cell infiltration, a massive increase in bacterial numbers, and tissue necrosis. These mortalities should probably be categorized as being of unknown etiology.

Undoubtedly the best documented, most studied, but least understood oyster mortalities in Japan began in Matsushima Bay and adjacent waters of Miyagi Prefecture about 1961 (Fig. 51). A series of papers published in the *Bulletin of the Tohoku Regional Fisheries Research Laboratory* in 1965 (Imai *et al.*, 1965; Kan-no *et al.*, 1965; Mori *et al.*, 1965a,b; Numachi *et al.*, 1965; Tamate *et al.*, 1965) described studies of mass mortalities of oysters that occurred annually in late summer. Environmental, physiological, and pathological factors were examined. Pathological changes were observed, and the cause of mortalities was believed, inconclusively, to be related to metabolic disturbances associated with spawning. An elaborate physiological explanation for mortalities of oysters in hanging culture in Matsushima Bay was proposed by Mori *et al.* (1965a,b, 1966) and by Mori (1967, 1968). The hypothesis was based on overmaturation of the gonad under the influence of high tem-

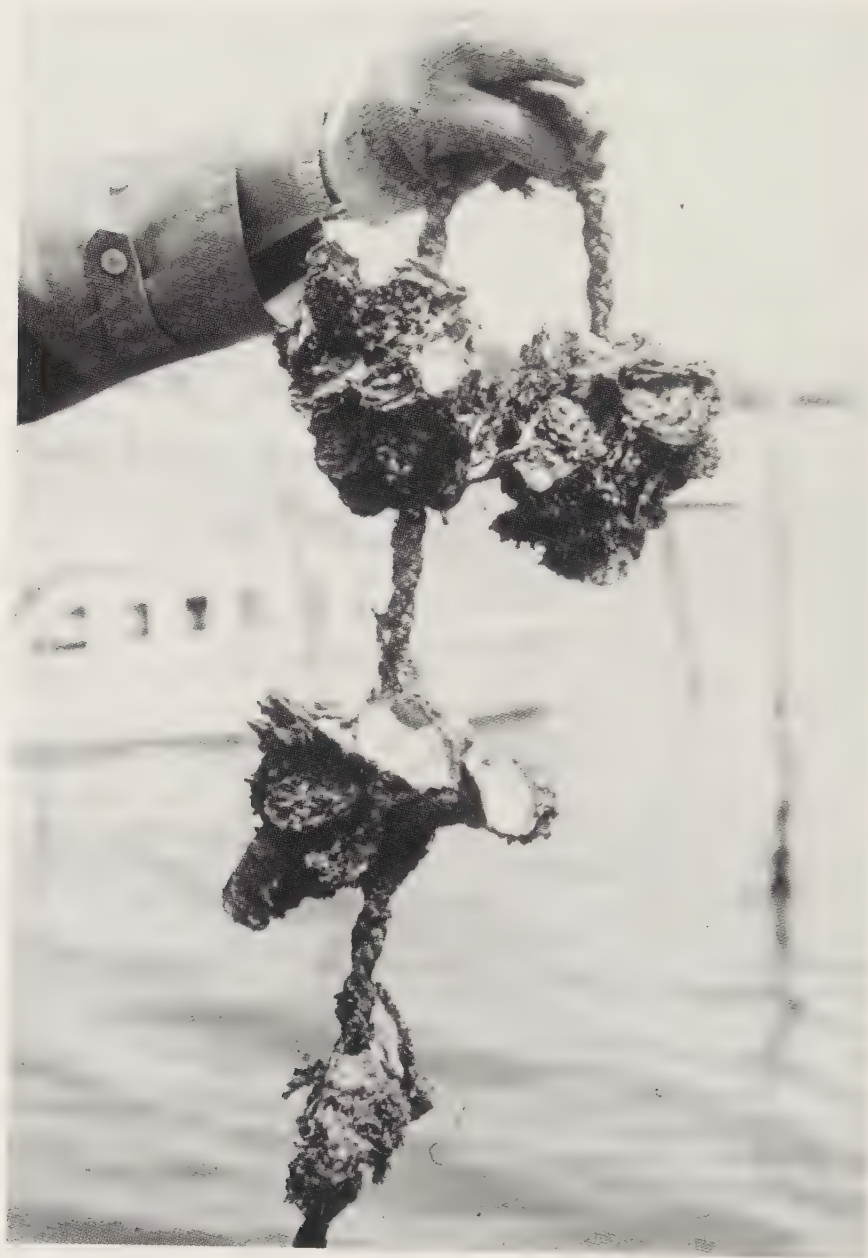


Figure 51. Oysters from hanging culture in Matsushima Bay. Empty shells indicating recent mortalities.

peratures and eutrophication of growing areas, disturbance of lipid and steroid metabolism, glycogen depletion, an extreme decline in physiological activity, and death.

Mortalities exceeded 60% per year in certain areas of the Bay during 1961–1965 and were selective, in that other bivalves did not die. Mature oysters in apparently good condition (by gross examination) died. A gram-positive bacterium was found in multiple abscesses in up to 20% of certain samples (Numachi *et al.*, 1965), but a causal relationship with mortalities was not thought to exist. Later studies by the Oxford (Maryland) Laboratory of the U.S. Bureau of Commercial Fisheries (now the National Marine Fisheries Service) suggested that the disease condition is the same as that called “focal necrosis” in adult Pacific oysters from the state of Washington and in Japanese seed oysters imported into Washington. The pathogen warrants further observation, because the abscesses may represent only the chronic stage of infection in more resistant hosts; the acute disease may be significant in causing mortalities.

The work of Japanese scientists involved with the Matsushima Bay mortalities has been of particular conceptual importance in that it deemphasized infectious disease and emphasized other, and in some instances new, possible causes of mortalities—particularly the physiological stress hypothesis described above.

Koganezawa (1975), in an informative summary, stated that mass deaths of oysters had occurred annually since 1961 in Matsushima Bay and since 1969 in other important oyster culture areas, particularly but not exclusively on the Pacific Ocean coast of Japan and the Inland Sea. Mortality rates were estimated at 40–60% annually. Koganezawa further pointed out the striking resemblance of earlier mass mortalities in Hiroshima Bay and more recent ones in Matsushima Bay. Characteristics in common were that mortalities occurred during and immediately following the spawning season, were greater in larger, faster-growing oysters, began when water temperatures reached 21°C, and were more severe when temperatures were high. He also pointed out that most of the major Japanese oyster production was in areas of advancing eutrophication, and he subscribed to the hypothesis of Mori (described above), which attributed mortalities to gross metabolic disturbances resulting from heavy, rapid gonad formation and massive spawning during times of high water temperatures in eutrophic bays. Infectious agents were not considered by Koganezawa to be the primary causes of mass mortalities in Japan.

Japanese oyster growers have adopted practices which prevent heavy gonadal formation and massive spawning, in an attempt to reduce mortalities. Koganezawa described two methods: (1) the use of

hardened seed oysters (exposed to winter air temperatures during part of the tidal cycle) and (2) placement of oysters in poor nutritional areas during the gonadal maturation period, and their removal to nutritionally rich areas for fattening in the fall and winter. Both methods prevent or postpone gonadal formation; it will be interesting to see if they also prevent mortalities.

Koganezawa (1975) made the additional observation that the development of intensive hanging culture methods for oysters in Japan has been accompanied by increasing mass mortalities. Emphasis on early fattening and harvesting and higher production in restricted areas may have contributed to the mortality problem. A paper from the Republic of Korea (Chun, 1970), where intensive culture was introduced in 1963, supported a possible association of this type of culture with mortalities. Comparing mortality rates of oysters grown by the traditional bamboo stake method with those grown in hanging culture, Chun found significantly higher mortalities in the latter (a peak of 37% in hanging culture in 1969, opposed to only 12% in bamboo stake culture).

3. Oyster Mortalities in North America

Oysters of commercial importance in North America include: the American oyster, *Crassostrea virginica*, grown on the Atlantic and Gulf of Mexico coasts; the Pacific oyster, *Crassostrea gigas*, introduced in the 1920s on the Pacific coast; and the Olympia oyster, *Ostrea lurida*, native to the Pacific Northwest. Trial introductions of the European oyster, *Ostrea edulis*, have been made on both the Atlantic and Pacific coasts, and are beginning to achieve commercial significance.

Oyster production in North America is still largely from natural beds, even though some rudimentary culture practices are carried on and even though some commercial shellfish hatcheries exist. Many of these natural beds have been affected by mass mortalities, some of extended duration, with long-term impacts on production.

Mortalities with a history of long and frustrating scientific study were first observed in 1915 in *C. virginica* from Prince Edward Island, Canada, in the Gulf of St. Lawrence (Needler and Logie, 1947). In the period from 1915 to 1939, the disease, called Malpeque Bay disease, spread around the island and destroyed most of the oyster stocks, some of which required 20 years to return to previous levels of abundance (Logie, 1956). The most obvious signs of infection were extreme thinness of meats, yellow-green pustules on the body surface, stunting of shell growth, and failure to spawn. About 95% of all oysters in an affected area were killed within 3 years of the first deaths. During the outbreak period oysters apparently developed resistance to the causative orga-

nism, whose identity remains undetermined. Recovery of a devastated stock by natural reproduction of resistant stock to a point at which it could support a fishery took 10–15 years (Logie, 1956). During this period severe selection pressure was demonstrated by high but declining mortalities of spat.

Beginning in 1955 mortalities (probably due to the same disease) began in waters of the adjacent mainland of New Brunswick across the Northumberland Strait from Prince Edward Island. Oyster populations along the entire northern coasts of New Brunswick and Nova Scotia were decimated within the next 5 years, but mass transfer of disease-resistant oysters from Prince Edward Island waters, beginning in 1957 and continuing until 1962, hastened the recovery of the fishery (Logie, 1958a,b; Logie *et al.*, 1960; Drinnan and England, 1965).

The great oyster mortalities of the Gulf of St. Lawrence have waned; recovery of stocks has been aided by transplantation, but control of the causative agent was achieved only by natural forces. If the mortalities were due to an infectious agent, and if that agent is still present (as it seems to be) in resistant stocks, then it is hard to understand why the identification of the disease organism has proved to be so difficult, in view of the intensive (if somewhat sporadic) investigations which were carried out. Today, more than 70 years after the mortalities began, the cause of the mortalities remains a mystery, even though numerous organisms have been offered as possible candidates.

Whereas the early oyster mortalities in Canada caused by Malpeque Bay disease have left scientists with a sense of frustration, investigations of recurring mortalities of *C. virginica* in the Gulf of Mexico beginning in the late 1940s have proved more definitive. Mackin *et al.* (1950) recognized and described a protistan parasite, *Dermocystidium marinum* (now called *Perkinsus marinus*) as the etiological agent. Exerting its effects in higher salinities and temperatures among dense aggregations of oysters, the pathogen has been reported to cause annual mortalities in excess of 50% (Ray *et al.*, 1953; Ray, 1954a,b; Ray and Chandler, 1955; Mackin, 1962). It has been identified in oysters throughout the Gulf of Mexico and northward along the Atlantic Coast as far as Connecticut (Hewatt and Andrews, 1956; Andrews and Hewatt, 1957; Andrews, 1965). Although prevalence of the pathogen may at times reach epizootic proportions in particular areas, as it has in recent years in Chesapeake Bay, its most significant effect is probably that of continuing attrition year after year during periods of high seawater temperatures. Effects of the disease on commercial beds have been controlled to some extent by planting and harvesting at prescribed times of the year and by planting oysters thinly on the beds.

Early discovery of a useful diagnostic technique, based on a fluid-

thioglycolate medium fortified with antibiotic, by Ray (1952, 1966a,b) enabled rapid determination of infection levels and distribution of the pathogen. *Perkinsus marinus* is common in most high-salinity waters of the southern United States. Ray (1966a,b) surveyed the occurrence of the fungus in the Gulf of Mexico in 1961 and in 1962, and found infections in 35 of 39 oyster samples, with prevalences as high as 100%. Hoese (1964) was able to find the organism in the digestive tracts and feces of fish, oyster drills, and crabs that had fed on dying and dead infected oysters. He speculated that transmission of the fungus might be furthered by scavengers, and that scavengers may release the parasite from host tissue.

The continuing role of *P. marinus* as a cause of mortalities has been investigated. A detailed study of the pathogen in Florida waters (Quick and Mackin, 1971) indicated that "the normal annual mortality from *P. marinus* parasitism is about 50% of the adult oyster population." Other studies have disclosed high prevalences of the pathogen in Texas, Mississippi, and Louisiana waters. Oyster culture schedules and planting densities have been adapted to reduce the impact of the disease. Methods used include: (1) low-density planting (since transmission of the pathogen is direct), (2) early harvest at minimal commercial sizes, (3) planting of large seed oysters in high salinities late in the growing season, and (4) utilization of low-salinity growing areas.

Major mortalities, with consequent severe depression of the oyster fishery, have also occurred in Delaware and Chesapeake bays on the U.S. East Coast, beginning in the late 1950s and continuing to the present (1989). A haplosporidan (ascetosporan) parasite with distinctive characteristics, *Haplosporidium nelsoni* (Fig. 52), has been identified as the cause of the mortalities. Extensive mortalities and a drastic decline of the oyster fishery began in Delaware Bay about 1957 and in lower Chesapeake Bay in 1959 (Haskin, 1961; Mackin, 1960; Engle and Rosenfield, 1963; Andrews, 1964, 1984; Farley, 1968). In each affected area mortalities exceeded 95% for several years.

As an extreme example of the effects of *H. nelsoni* on U.S. oyster production, landings in New Jersey waters of Delaware Bay in the late 1940s and the early 1950s had fluctuated around 6 million pounds (2724 metric tons) of shucked meats. Disease decimated the stocks beginning in 1957. Landings fell precipitously to a low of 167,000 pounds (75 metric tons) in 1960, and have not recovered significantly (Fig. 53). Recent statistics disclose landings still under 1 million pounds (454 metric tons). The disease is still (as of 1989) at epizootic levels in Delaware Bay, but, apparently because of increased resistance, disease-caused mortality is only at 50% of its original level (Ford and Haskin, 1982). A cyclic pattern

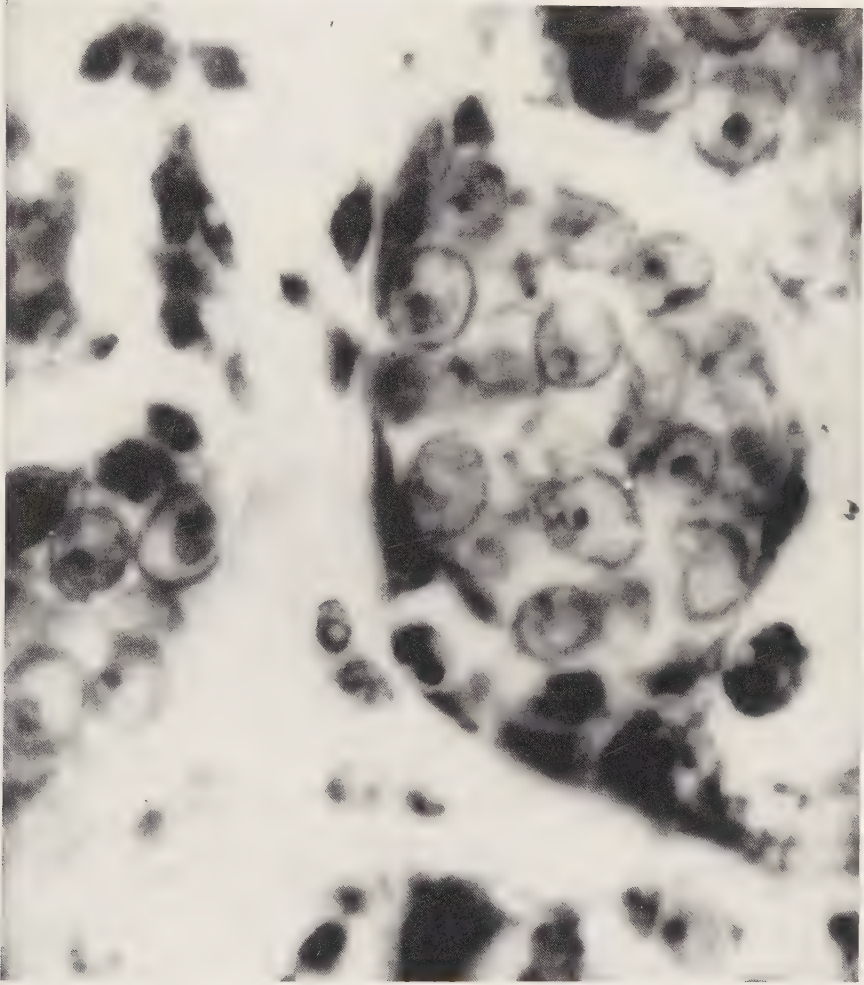


Figure 52. Spores of *Haplosporidium nelsoni* in tissues of *Crassostrea virginica*.

of infections has been discerned, with peaks every 6–8 years. It has been estimated that at least half of all oyster deaths in lower Delaware Bay since the early 1960s were related to the disease caused by *H. nelsoni* (Ford and Haskin, 1982).

Similar effects were seen in the high-salinity waters of Chesapeake Bay, another major oyster-producing area. The grim story of the impact of Delaware Bay disease on Chesapeake Bay oyster production was summarized by Andrews and Wood (1967): “. . . 1960 was the year of

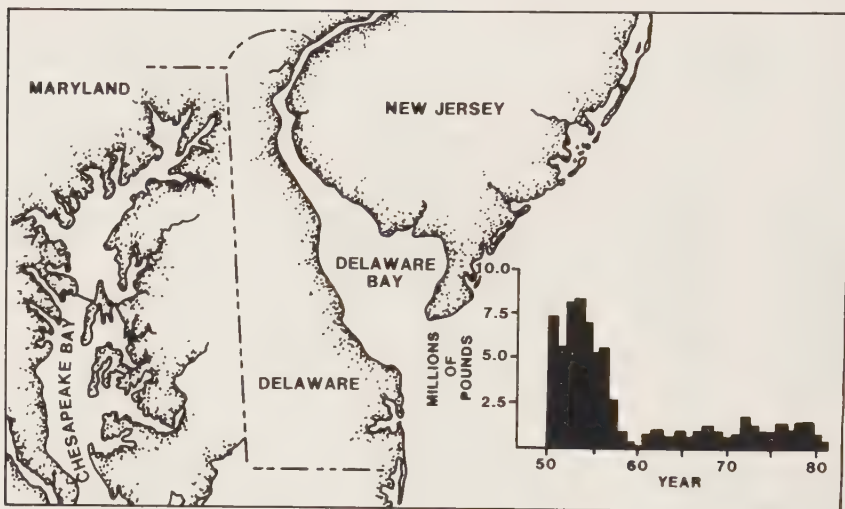


Figure 53. Oyster production in Delaware Bay, as affected by the *Haplosporidium nelsoni* epizootic (1 million pounds equals 454 metric tons).

reckoning for the industry. Late winter losses were followed by near decimation in early summer of plantings made before June 1959. In 1960 the last commercial plantings were made in lower Chesapeake Bay and oysters were decimated in a vast area. By June 1961, all plantings worth salvaging had been marketed, regardless of age. Millions of dollars worth of oysters were lost in 1960. . . ."

High salinities enhance disease effects. *Haplosporidium nelsoni* occurs in waters whose salinity consistently exceeds 15 ppt. During 1963–1965, with low rainfall along the Atlantic coast, the pathogen invaded areas of middle Chesapeake Bay, formerly free of the disease (Rosenfield and Sindermann, 1966). Since that time the disease has progressed further northward in the bay, exerting severe effects and expanding its distribution in drought years, when freshwater runoff is low and salinities are high.

Whether the pathogen was newly introduced into waters of the Middle Atlantic states or was enzootic in *C. virginica* populations of the area has not been determined. Circumstantial evidence favors the view that *H. nelsoni* may have been enzootic in the oyster stocks years before the epizootic (Andrews and Wood, 1967; Andrews, 1966, 1968). It is perhaps pertinent, however, that organisms similar to *H. nelsoni* have been reported as rare parasites of Pacific oysters (*C. gigas*) in the state of Washington, the Republic of Korea, and Taiwan (Pereyra, 1964; Sindermann and Rosenfield, 1967).

At the beginning of the major East Coast oyster mortalities caused by *H. nelsoni* in the late 1950s, a second ascetosporan (haplosporidan), *Haplosporidium costale*, was identified as the cause of simultaneous, but geographically restricted mortalities. The organism was found in seaside bays of Maryland and Virginia, along the lower eastern shore of Virginia, and in Delaware Bay (Andrews *et al.*, 1962; Wood and Andrews, 1962; Sprague, 1963; Haskin *et al.*, 1966; Couch, 1967). First recognized in moribund and dead oysters from Hog Island Bay, Virginia, by Wood and Andrews (1962), *H. costale* was considered to be responsible, on the basis of epizootiological evidence, for sharp peaks of mortalities in May and June, with a sudden onset of mortalities in May and abrupt termination of mortalities about mid-June. The pathogen and mortalities caused by it continue to affect Maryland and Virginia seaside oyster populations (Couch, 1967; Couch and Rosenfield, 1968; Andrews and Castagna, 1978; Andrews, 1984).

While mortalities of *C. virginica* were occurring on the U.S. Atlantic coast in the late 1950s and the 1960s, similar events were taking place in *C. gigas* populations on the U.S. Pacific coast. *Crassostrea gigas* at that time was largely imported as seed from Japan and planted in waters of the West Coast states of Washington, Oregon, and California. Mortalities were most serious in the two principal West Coast growing areas, Puget Sound in Washington and Humboldt Bay in California (Katkansky and Warner, 1974). The condition was labeled "summer mortality." Oysters in their second year after introduction were most commonly killed, and deaths were often noted at the heads of bays, although occurrences were irregular. Unlike the East Coast mortalities, no clear association was made of specific pathogens with mortalities of *C. gigas* on the Pacific coast of the United States, and it is quite possible that other environmental factors were operative in the mortality areas. (It should be noted, though, that bacterial infections were observed in some instances.)

Glude (1975) published an excellent account of the West Coast oyster mortalities and the attempts to understand them. Losses in principal growing areas reached 35–58% annually during the 1960s. He also found that oysters died during the second summer following planting; most seriously involved were fast-growing individuals near the heads of eutrophic bays and estuaries in Puget Sound and in much of Humboldt Bay. Glude emphasized the striking similarities between mortalities in Matsushima Bay, Japan, and those in Puget Sound. In both places, as he pointed out: (1) fast-growing oysters died, (2) affected oysters were characterized by rapid overmaturation of the gonads, (3) mortality occurred during the summer spawning period, and (4) no specific pathogen could

be associated with the mortalities, despite indications of degenerative necrosis of the digestive diverticulum.

In an attempt to understand the causes of the West Coast mortalities, a large, federally funded cooperative investigation was carried on from 1966 to 1972. This resulted in an unparalleled description of the pattern of oyster mortalities, related environmental events, and histopathological observations. One of Glude's conclusions was that "The failure of pathological investigations to find a causative organism tends to strengthen the physiological stress theory" (discussed earlier in this chapter, as Mori's hypothesis to account for Matsushima Bay mortalities). The termination of federal funding in 1972, with a consequent reduction in the research effort, coincided with a decreasing trend in mortalities observed during 1972 and 1973 (Glude, 1975). This agreed generally with the finding of Sindermann (1975) that, with localized exceptions, the West Coast mortalities subsided (but did not disappear) after about 1970.

Interest in the problem of summer mortality of *C. gigas* did not subside, however. Studies at the University of Washington in the 1970s demonstrated experimentally that mortalities could be induced by raising water temperatures above 18°C (Lipovsky and Chew, 1972), and that mortalities were associated with physiological stresses of gametogenesis and glycogen depletion (Perdue *et al.*, 1981). Additionally, a program was started in the early 1970s to produce, by selection, strains of oysters that were resistant to the conditions. Since mortalities seemed to be temperature related, temperature stress was used to select mortality-resistant oysters (Beattie *et al.*, 1980). After three generations mean mortality for selected groups ranged from 13 to 23%, whereas that of the control group was 62% (Hershberger *et al.*, 1984). Survival was tested on commercial grow-out grounds in areas known to be subject to mortalities.

4. Oyster Mortalities in Australia

Reports of oyster mortalities in Australia extend back to 1890, when Whitelegge published a report on worm disease caused by the annelid *Polydora* in New South Wales. He considered the disease (with limited evidence) to be a cause of extensive mortalities, which eventually brought about the development of intertidal culture methods in New South Wales.

Commercial oyster production in Australia is restricted largely to New South Wales, and to the Sydney rock oyster, *Saccostrea commercialis*. Throughout much of the 20th century, an important factor limiting production has been the so-called "winter mortality." First described in the

scientific literature by Roughley (1926), the mortalities had characteristics very similar to those seen in European *Ostrea edulis* during the extensive mortalities of 1919–1923. Roughley described mass deaths of oysters in the Georges River, New South Wales, in 1924 and in 1925. Disease signs, in the form of abscesses and ulcerations, were observed, and a bacterial pathogen was suspected but not demonstrated. Winter environmental stress was also implicated, since rock oysters are grown intertidally.

Wolf (1967) reviewed the history of winter mortality, concluding that the cause was still essentially unknown, but salinity may be a factor, since high mortality occurred in years of low autumn rainfall. Bacterial infections may be involved, possibly aggravated by winter stress. Wolf reported that annual and areal effects may vary, but severe outbreaks of winter mortality can kill 80% of the marketable oysters in specific production areas and can affect younger oysters as well. Signs in affected oysters include pinhead ulcerations in the flesh, first in the palps, then in the gills, and finally in the adductor muscle and the gonad. Oysters then gape and die. Sprague (1971) pointed out the similarity of these signs to European gill disease, caused by an iridovirus.

Beginning in the late 1960s, quite a different type of mortality occurred in *S. commercialis* in Moreton Bay, Queensland, Australia, and persisted for several years (Wolf, 1972). A sporulating ascetosporan, *Marteilia sydneyi* (Perkins and Wolf, 1976), was seen in the tissues of moribund oysters and was considered to be responsible for the mortalities. This finding is significant, in view of the recent oyster mortalities on the U.S. East Coast caused by ascetosporans and the recognition of similar organisms in oysters from the U.S. West Coast and from Korea. The Australian oyster pathogen is in the same genus as the parasite associated with digestive gland disease of the European *O. edulis* (i.e., *Marteilia refringens*).

5. Oyster Mortalities in the Pacific Islands

Mortalities in native and introduced oysters have been reported from islands of the South Pacific. Introduced *C. virginica* in Pearl Harbor, Hawaii, died during an epizootic of a *Perkinsus* sp. in the summer of 1972 (Kern *et al.*, 1973). Mortalities exceeded 90%, and the high prevalence of a *Perkinsus*-like parasite was confirmed by culture and by histological studies.

Crassostrea gigas in hanging culture in Fiji died in 1975 and in 1976, with mortalities reaching 88% (R. M. Stone, personal communication to J. B. Glude, 1977). No pathogen was identified, and the cause was felt to

be environmental stress. Similar mortalities of *C. gigas* in their second year of life occurred in Palau (J. B. Glude, personal communication, 1977).

Mortalities occurred in native mangrove oysters of French Polynesia, *C. glomerata*, in the early 1970s (J. B. Glude, personal communication, 1977). Histological examination did not disclose a pathogen.

All of these island mortalities are, of course, localized and do not approach the dimensions of the mass deaths discussed in previous sections.

B. Control of Oyster Mortalities

Mortalities in natural oyster beds are not usually subject to control in the sense that populations in restricted culture environments may be controlled (e.g., by water quality management and the application of chemotherapeutic agents). What is feasible, however, is limitation of the extent of mortalities by environmental manipulation (e.g., cleaning beds of dead shells, selective removal of possible intermediate or reservoir hosts of pathogens, selective use of chemicals) or stock manipulation (e.g., moving oysters to low-salinity growing areas, planting at prescribed low densities, suspending oysters at specific depths in the water column, moving oysters to waters with poor nutrient content for part of the growing season, planting seed late in the growing season, and harvesting oysters before annual mortalities are expected). In only one instance has chemical control been proposed; Korringa (1952) suggested the disinfection of oysters with shell disease with organic mercury salt solution. (Because of its toxicity to other organisms, use of mercury would not be recommended at present.)

Usually, though, by the time effective control measures can be implemented, the peak of mortalities has passed and death rates have in some instances begun receding to background levels. Control methods can still be useful, however, in reducing that part of background mortality that is attributable to the long-term effects of specific pathogens. For example, control methods developed in the Gulf of Mexico for *Perkinsus marinus* epizootics (e.g., reduced stocking densities, use of low-salinity grow-out areas, and early harvest) are of continuing utility in suppressing mortalities in that area due to the pathogen.

Studies of two serious pathogens of oysters, *P. marinus* and *H. nelsoni*, have revealed the very important role of a "salinity barrier" to certain diseases. *Perkinsus marinus* exerts severe effects on oyster populations in high-salinity waters of the bays along the Gulf of Mexico coast, but does not flourish in low-salinity areas. *Haplosporidium nelsoni*, which

has seriously affected oyster stocks of the Middle Atlantic states, also prospers in higher salinities. Both pathogens seem to be most active in salinities above 15 ppt; this fact has made possible the continuation of production in parts of coastal areas affected by the epizootics.

Inhibitory effects of low temperatures have been well illustrated for these serious oyster pathogens. *Perkinsus* causes warm weather mortalities in American oysters; in fact, planting seed oysters is timed to take advantage of the relative quiescence of the disease in cooler seasons. Surveys of *Perkinsus* prevalences have indicated a marked decline in winter. *Haplosporidium nelsoni*, of Chesapeake and Delaware bays, is similarly quiescent in winter; new infections are not apparent, the prevalence of disease declines, existing infections seem less active, and mortalities are reduced.

The development of resistance to particular infectious agents can be used as a method of control. Severe mortalities exert selection pressure favoring survival of individuals with some degree of resistance. Propagation of the offspring of such individuals and reexposure to the pathogen over a number of generations can lead to a stock which is resistant to at least the acute stage of infection. Such a sequence of events seems to have occurred naturally in oysters of Prince Edward Island during the Malpeque Bay disease outbreaks. Resistance seems to have been developed also in oyster stocks exposed and reexposed to *H. nelsoni* in Delaware and lower Chesapeake bays. Here infection levels have remained high, but mortalities have declined. Experiments have been under way in Virginia and New Jersey to propagate artificially, using hatchery methods, oysters resistant to *H. nelsoni*.

Another approach to problems of mass mortalities is the *replacement* of a susceptible species with another which is more resistant to pathogens identified as causes of continuing mortalities. This approach was used on a massive scale in France (Deltreil, 1973) where *C. gigas*, imported as seed from Japan, reproduced successfully and has largely replaced *C. angulata* in most French oyster-producing areas.

The control of mortalities may, of course, depend on special requirements of the pathogen. The finding that a large reservoir of the fungus responsible for shell disease of oysters existed in dead cockleshells led Korringa (1951a,b) to propose, very logically, the removal of all dead shells from growing areas and the near vicinity, and to concentrate spat production in areas not heavily infected.

A final method to reduce mortalities is to retreat from the natural environment and grow oysters in closed systems, where environmental variables, including disease, may be controlled more effectively.

Since mass mortalities of oysters will undoubtedly occur in the future, as they have in the past, some measures useful in responding to the next major event should be examined:

1. Governments should institute a continuing monitoring system for coastal species of economic concern, such as oysters. This monitoring should include, but not be limited to, surveillance of prevalences of known pathogens and predators.
2. Some form of rapid funding response to marine environmental crises (such as mass mortalities) should be available. Even limited emergency funds can serve as a springboard for expanded research contributions by states (or provinces), universities, and industry.
3. Coordination of research efforts is important, without inhibiting individual contributions by experts. Ingredients of such coordination include a steering committee and an ad hoc advisory committee from the industry segments being affected.
4. The assembled research group should include as many disciplines as possible. Since a number of mass mortalities of oysters have been associated with pathogens, pathology obviously must have significant representation. However, environmental and physiological aspects must not be ignored, since mass mortalities can have complex etiologies.
5. As quickly as possible, and even before adequate research information is available, empirical attempts to find ways to alleviate effects of mortalities should be instituted. These may take the form, for example, of placing lots of oysters in different salinities, at different depths or in different densities.

C. Conclusions about Oyster Mortalities

The 20th century has been a troublesome period for oysters in many parts of the world. The decline in the abundance of oysters actually started in the 19th century, probably caused in large part by a combination of poor setting, indiscriminate harvesting, and destruction of beds. Extensive mortalities from unknown causes also contributed to decreased oyster production. The rate of decline has accelerated within the past two decades, partly because of large-scale mortalities, several of which have been caused by infectious diseases. Due to their worldwide economic importance, oysters are among the most thoroughly studied of marine animals, but unresolved problems remain. Mortalities constitute one such problem area.

Oysters die for many reasons. Mackin (1961) attempted to classify types of mortalities based on the following causes: (1) extremes of physical environmental factors, (2) disease, (3) starvation, (4) spatial competition, (5) predators, (6) toxins, and (7) metabolic competition. Certain general characteristics can be associated with each type. Unfortunately, an additional and very large category, "those of unknown origin," is the one which, as Mackin admitted, has "produced the most spectacular declines in populations of oysters." Probably, there should be still another category—"those of apparently complex origin"—since it seems that at least some of the recent mortalities in Japan and on the west coast of the United States may involve environmental and physiological components, with the possibility of disease as a secondary factor. Mackin concluded, with some justification, that of all of the causes of mortality, disease ranks first—both as a cause of spectacular major declines in production and as a cause of much background mortality.

Mass mortalities are receiving greater scientific attention than they did in the past. For example, oyster mortalities in the 1960s in Matsushima Bay, Japan, resulted in a combined research effort by the Oyster Research Institute, the Prefectural Fisheries Cooperative, the Tohoku Regional Fisheries Laboratory, and Tohoku University. Almost simultaneously, a large federally funded cooperative research group consisting of state, university, and federal research scientists was organized on the Pacific coast of the United States to investigate oyster mortalities occurring there. Both the Japanese and the American groups represented models for structuring research response to a severe mortality problem, since they included expertise in environmental as well as pathological specialties and provided for long-term cooperation in research.

What is needed badly—as pointed out by Sprague (1971) as well—is *continuity of research*, during quiescent periods as well as mortality periods. Examining mass mortalities while they are occurring can be an exciting experience, but the studies can be more useful if a chronicle of events preceding and following the mortalities is available. Insights from research often accumulate very slowly, sometimes long after oyster populations have been decimated and the public has lost interest in the problem.

In addition to continuity of research, a *critical mass of scientific competencies* is needed to approach effectively any problem of mass mortality. Expertise in biology, chemistry, ecology, pathology, immunology, and physical oceanography must be available initially, until some insights are gained which can give more precise direction to the investigations. It is very encouraging that during the past two decades there has been just

such a multidisciplinary examination of major mortalities that occurred in Europe, Japan, and the United States.

Looking at the record of successes and failures in attempts to understand mass mortalities, it is not difficult to become discouraged. Often these "visitations" have appeared, exerted a major impact on oyster production, then dwindled away, while individuals or small groups of scientists have groped in vain for causative factors. This course of events has been true for about half of the major oyster mortalities in this century. However, there is some solace in those instances (such as the *Perkinsus marinus* research in the United States) when causative organisms were recognized, understanding of the life cycle was developed, and reasonably effective control measures were proposed. There is greater solace in the obvious fact that our record of success in identifying causes of mortalities is improving dramatically. Disease agents have been recognized, classified, and tested for pathogenicity with increasing frequency in recent decades—in those instances when disease is a primary factor in mortalities.

Even though adequate insights about the origins of some earlier mortalities are still lacking, there have been enough investigations, particularly during the past two decades, to allow certain generalizations to be made:

1. Not all mortalities of oysters are caused by the activities of pathogens, but among the disease organisms identified as etiological agents of epizootics and concomitant mass mortalities there are two protozoan groups—the ascetosporans (haplosporidans) and the apicomplexans—which are emerging as being of worldwide significance. The *Perkinsus*-like organisms in particular may be of much greater significance to oyster populations than has been demonstrated. In addition to numerous recent reports of such organisms in European oysters, there is also the observation by Quick and Mackin (1971) of a lethal oyster parasite—possibly a second *Perkinsus* species—associated with oyster mortalities in Florida which were originally thought to be caused by *P. marinus* alone.
2. During times of mass mortalities, there is often pressure to assign a cause. The identification of a parasitic organism in the tissues of oysters is an important but very preliminary phase in the process of assigning blame for mortalities. The rigorous sequence of steps necessary to associate a pathogen with a disease is difficult to accomplish with marine organisms, particularly if the pathogen is not easily cultured. Without these steps, it is possible to reach erroneous conclusions.

3. *Mass mortalities may affect any stage in the life history of the oyster, from larva to adult.* The early life stages are very vulnerable: long experience in shellfish hatcheries (Davis *et al.*, 1954; Tubiash *et al.*, 1965) has demonstrated that larval mortalities are not uncommon; other studies have demonstrated the high mortality rate of spat in the first year of life (Walne, 1961; Westley, 1975). Walne found in a 6-year study that mortality of *Ostrea edulis*, in the first year of life after setting, varied between 66 and 92%. Westley was concerned with the fate of seed oysters, *Crassostrea gigas*, planted on growing beds in Puget Sound, Washington. He found mortalities of about 62% during the first year after planting (partly due, of course, to smothering of seed on the underside of the cultch). On the other hand, very young oysters seem to be less vulnerable than older ones to mortalities caused by pathogens such as *Perkinsus* or *Haplosporidium*—at least during the first few months of life.

Mackin (1961) attempted, from a review of the literature existing at that time, to itemize the characteristics of mortalities of oysters due to various causes. As one who published extensively on the role of disease in oyster populations, he naturally turned his attention to mortalities caused by infectious agents. He concluded that disease can cause significant, if temporary, reductions in the population abundance of marine invertebrates. Such reductions may exceed 95% of existing stocks. Additionally, there is every indication that serious but undescribed diseases exist among marine invertebrates. Mackin (1962), for example, mentioned a number of pathological conditions in oysters that were not associated with known pathogens. Disease conditions are often recognized by fishermen, but have not been described in the published scientific literature.

4. *If we sift through enough literature on principal diseases of molluscs—Haplosporidium nelsoni disease, Perkinsus marinus disease, clam neoplasia—we can begin to see evidence for a general epizootic pattern for molluscan diseases.* The process begins with an initial focus, followed by a rapid spread of infections and a rapid rise in mortalities, in which most of the population (>95%) is lost. This is followed by a number of years of gradually decreasing but still substantial levels of disease and mortalities. During this dismal period of pestilence and death, the slow emergence of signs of resistance can be discerned, in the form of remissions and fewer mortalities among the pitifully scarce survivors. Just when things seem to be improving, though, repeated peaks of mor-

talities recur, some with a suggestion of periodicity (but none with peaks as high as the initial mortalities). By then, research programs have provided experimental demonstration of pathogenicity and lethality, and sometimes information on empirical methods to mitigate effects of the disease.

In all of these outbreaks and their sequelae, the significant role of environmental factors is apparent—especially the dominant ones of salinity and temperature (and their variations). Finally, after several decades of intense infection pressure and selection, we emerge on a plateau of uneasy compromise—when the disease still lurks in the host population but mortalities have subsided to “acceptable” levels (i.e., levels at which marginal commercial production can exist if appropriate control measures are taken).

Molluscs seem to function with an “operating base” different from that of the vertebrates (and possibly even that of the larger Crustacea), in that *primary investment is in an altered population genetic response to a pathogen rather than in an elaborate protective system of acquired immunity against specific pathogens*. In practice, this implies (even requires) mass mortalities, in which over 95% of the existing populations disappear, as do most of their offspring, nudging population resistance ever so slightly in each generation in a direction favoring increased survival of future generations.

III. EPIZOOTICS AND MASS MORTALITIES OF CRUSTACEANS

Epizootics of infectious diseases, with accompanying mortalities, have been a matter of record in natural populations of crustaceans or more than a century. Disease outbreaks have occurred in the larger commercial Crustacea, in the smaller food chain species, and more recently in aquaculture populations. There is some evidence that the larger Crustacea are immunologically responsive and that environmental factors—notably aquatic pollutants and poor culture conditions—can influence the course and the severity of disease outbreaks. There is also good evidence for a significant influence of virulent primary pathogens in affecting the abundance of crustacean populations.

Mass mortalities of crustaceans are often difficult to assess because:

1. Sick or moribund individuals are secretive and behave abnormally, so they may be underrepresented, if represented at all, in samples.
2. Sick animals cease feeding and do not enter baited traps (which are the usual sampling devices).

3. Sick animals may be lethargic or may have abnormal behavior or coloration, which makes them more vulnerable to predators.

Despite these limitations, information about the effects of epizootics on crustacean populations has been acquired, and some of it is summarized in this section.

No examination of epizootics and mortalities in crustacean populations—even in a book on *marine* diseases—can logically start anywhere but with the great crayfish plague, *krebspest*, which began in Italy in about 1860 and decimated much of the freshwater crayfish (*Astacus astacus* and *Austropotamobius pallipes*) populations of Europe during the ensuing 100 years (Ninni, 1865; Leuckart, 1884; Seligo, 1895; Weber, 1899; Schikora, 1903, 1926; Nybelin, 1935, 1936; Schäperclaus, 1935; Pixell-Goodrich, 1956; Unestam, 1964, 1969a–c). Caused by the oomycete fungus *Aphanomyces astaci*, mortalities in some areas reached 100%, completely eliminating many crayfish populations.

The sequence of events during the spread of infections was well described by Unestam (1973): “The first appearance of the disease in Italy in 1860 was reported by Seligo (1895). Later it spread through France and central Europe and before the turn of the century it had reached deep into Russia and north to Finland. In 1907 it reached Sweden, where now more than 50% of the crayfish waters have lost their delicate decapods because of the disease, which is still spreading slowly from lake to lake. Also in eastern Europe the disease is continually exterminating additional populations of crayfish (Mannsfeld, 1942; Cukerzis, 1964; Kozlowski, 1968).”

Crayfish plague is still extant in Europe, and it continues to invade previously unaffected populations. The disease was reported definitively in Britain in 1981 (Alderman *et al.*, 1984); total mortalities of crayfish populations have since occurred in a number of rivers in southern England.

It is interesting, in reading the early and massive literature on *krebspest*, to see the sequence of events that has been repeated often during modern epizootics in aquatic populations.

1. A primary focus of disease occurs (in this case, in Italy) but the disease quickly spreads to other areas (throughout Europe).
2. There is an extended period when the etiological agent is unknown, or several pathogens are claimed separately as the etiological agent (in this case, a bacillus was in the lead, and numerous other bacteria were blamed, even though the true culprit, the fungus *Aphanomyces astaci*, was named and identi-

fied by Schikora in 1906). Public arguments over favored pathogen candidates develop, often with much vituperation and scientific name-calling.

3. As the epizootic affects new geographic areas, many new people become involved, and a few of these stay involved, to produce significant new information (in the case of the crayfish plague it seemed to be up to German workers principally—first Leuckart and Weber, then Schikora, and then Schäperclaus—with contributions from Swedish researchers—Nybelin and, more recently, Unestam and other contemporary scientists).

Some aspects of the epizootiology of the fungal pathogen *A. astaci* are very significant:

1. The European crayfish seems to have no resistance to the fungus, so mortalities in newly invaded areas are 100%.
2. In affected waters no crayfish survive, so no resistant individuals exist to form the nucleus of a resistant population.
3. No resistant spores of the fungus have been found, so new populations of crayfish may be introduced into previously affected areas after the original populations have disappeared.
4. Experimentally, infectivity of the pathogen is high. Only a few zoospores per milliliter are sufficient to infect European crayfish.
5. All U.S. species of crayfish are resistant, but can be infected experimentally if exposed to many spores. The disease is quickly circumscribed in these species, causing localized nodules. Hyphae are melanized rapidly, in the muscle and in the cuticle.

From the history of the spread of *krebspest* throughout Europe, and its devastating effect on populations of indigenous species there, it is legitimate to conclude that it was introduced to Europe, probably from the United States. Evidence to support this hypothesis was stated succinctly by Unestam (1973):

All the 4 European crayfish species, belonging to the genera *Astacus* and *Austropotamobius*, seem equally susceptible to the epizootic (Schikora, 1916; Unestam, 1969a). The same is true of the only Japanese species, *Cambaroides japonicus*, and possibly also of the only tested Australian crayfish, *Euastacus kershawii* (Unestam, 1969a, 1972), but in Japan and Australia the plague has never appeared. The complete lack of any resistance in all these crayfish groups indicates that the pathogen does not belong on them as an adapted parasite. In North America, however, three different groups of crayfish (one related to the European groups) showed high but not complete resistance (Unestam, 1969a; Unestam and Weiss, 1970). The fungus was also

found as a natural parasite on an American species, *Pacifastacus leniusculus*, to which it probably causes little harm (Unestam, 1972). The fungus is easily grown in pure culture (Rennerfelt, 1936) and shows strong physiological adaptation as a parasite on freshwater crustaceans but pathological adaptations to only decapods (Benisch, 1940; Unestam, 1969a,b). The North American crayfish groups appear to be adapted either to survive in the presence of the parasite or to be completely resistant to it. Resistance in these groups, which are not closely related, may therefore have developed in parallel but independently (Unestam, 1969a).

Just as we must accord the European crayfish plague a place of honor in any consideration of crustacean epizootics, so also must we pay respects to gaffkemia of American and European lobsters, caused by the micrococcus *Aerococcus viridans* var. *homari*.

Gaffkemia, or red tail disease, as it was originally called, was first recognized on the Maine coast in 1946. Two epizootics of gaffkemia occurred—affecting lobster populations of the entire Maine coast—in 1946–1947 and in 1959–1960 (Goggins and Hurst, 1960). Mortalities during the epizootic years were especially apparent when attempts were made to impound lobsters, a common practice in Maine, Canada, and elsewhere. Losses in such impounded populations regularly reached 60% within a few weeks.

In addition to the widespread epizootics of 1946–1947 and 1959–1960, there have been scattered reports of more localized mortalities possibly related to gaffkemia. One such event occurred in Seabrook Harbor, New Hampshire, in 1977 (Hickey, 1978). Although several other environmental factors may have contributed to mortalities, *A. viridans* was found to be present in samples of dead lobsters from the mortality area. In investigating the New Hampshire mortality, Hickey found that localized gaffkemia problems occurred almost every year in lobster holding facilities somewhere along the coast of Maine, as reported by the Maine Department of Marine Resources.

There is some suggestion in the literature that gaffkemia has killed impounded lobsters on the east coast of North America since that practice of the industry began. Herrick (1909), for example, described a mortality of about 100,000 market-sized animals being held at Portland, Maine, in 1893. Rapid onset and other characteristics suggested a disease outbreak, even though the etiology was not determined.

The disease is also well known in European lobster populations (in which epizootics have been reported in England, Ireland, Norway, and The Netherlands). Mortalities of European lobsters, *Homarus gammarus*, from *A. viridans* have been known and reported for more than 30 years. In fact, the common name “gaffkaemia,” was used originally by Roskam

(1957) in describing disease-caused mortalities in impounded lobsters in The Netherlands, which began in 1955.

Factors of significance in the epizootiology of gaffkemia include:

1. Lack of any observable resistance of lobsters to infection
2. The apparently heterotrophic capabilities of the pathogen in bottom muds and in seawater
3. Direct transmission of the pathogen
4. Direct temperature dependence (mortalities increase sharply when water temperature exceeds 15°C)
5. A vanishingly small infective dose (as few as five organisms per lobster produced 90% mortality in experimental studies)
6. Infectivity of the pathogen for other crustacean species (it does not cause epizootics in these other species, but they may act as reservoir hosts)
7. Transmission through ruptures in the integument

Gaffkemia undoubtedly contributes to continuing background levels of mortality in lobster populations, in addition to causing periodic mass mortalities. Surveys have disclosed sometimes significant prevalences in wild populations, even when mortalities have not been reported, but it is in captive populations in commercial holding operations that the most severe effects are seen (Stewart, 1984). This is because:

- Animals are handled roughly throughout capture and holding, so wounds and abrasions are common.
- Holding conditions (especially low dissolved oxygen and high BOD levels) are often marginal, and the animals, which are naturally aggressive, are usually crowded.
- The pathogen is free living, so it can survive in mud and detritus, or on holding cages and plumbing, for months.
- Sterilization of holding facilities following an outbreak is extremely rare.
- Water temperatures in holding facilities may be higher than in the natural environment, thus increasing the severity of outbreaks.
- The pathogen is able to infect and survive for extended periods in a wide range of crustaceans, including species such as the rock crab, *Cancer irroratus*, and the Jonah crab, *Cancer borealis*, which inhabit the same area as the lobsters.
- Claws of captive lobsters are immobilized by pegs, which penetrate the exoskeleton and provide an invasion route for bacteria, including *A. viridans*.

Surveys of gaffkemia in Canadian Atlantic waters disclosed widespread distribution in all areas sampled, with an overall prevalence of almost 5%, but with marked geographic variability (Stewart *et al.*, 1966). These figures are undoubtedly conservative, since diseased individuals cease feeding within 2 days after initial infection and are then unlikely to enter baited traps to be counted (Stewart, 1984; Sparks, 1985).

A third example of epizootic disease with resultant mass mortalities occurred in blue crab (*Callinectes sapidus*) populations in high-salinity waters of the Atlantic coast of the United States from Maryland to Florida in the late 1960s and the early 1970s. Called "gray crab disease" because of a characteristic discoloration of the ventral exoskeleton, the disorder is caused by a sarcomastigophoran protozoan, *Paramoeba perniciosa* (Sprague and Beckett, 1966, 1968; Sprague *et al.*, 1969; Sawyer, 1969; Newman and Ward, 1973; Johnson, 1977). Mortalities occurred in wild populations and in crab holding tanks, the course of the infection was progressive and rapid, and all crabs with patent infections died. Detailed study of one crab-producing area, Chincoteague Bay in Virginia, resulted in an estimate that one third of the commercial crab population was killed by the disease in a single month (June 1971) (Newman and Ward, 1973). These authors speculated that gray crab disease may have been responsible for previously reported mass mortalities of blue crabs in other Atlantic coastal areas. High prevalences of infections (up to 20%) and mortalities were reported in early summer and again in the winter, during the crabs' hibernation period (Couch, 1983).

Annual variations in the abundance of gray crab disease have been noted, with outbreak periods alternating with years when mass mortalities are not detected, although chronic, low-level losses may occur in those years when the disease is less apparent. Infections seem correlated with the summer molting period of blue crabs, and losses to the shedding (soft crab) industry can be substantial (Couch, 1983).

The epizootics described in European crayfish, American lobsters, and blue crabs have occurred in wild populations of economic significance—but what about all the smaller Crustacea important as food chain organisms (the copepods in particular)? Is there any evidence for epizootics and mass mortalities in such species? Some information exists, although it is scarce when compared with that available for the larger commercial species. One example of the fact that epizootics can occur in food chain populations was called to my attention by Dr. A. Fleminger of the Scripps Institution of Oceanography in 1964 (personal communication). He had found in plankton tows a significant prevalence (about 15%) of systemic fungus infections in calanoid copepods of the open Pacific. The parasite was morphologically similar to *Ichthyophonus*, which had

been described earlier (Jepps, 1937), in copepods in North European waters.

A better example of disease and associated mass mortalities of copepods was reported by Vallin (1951). An epizootic in populations of *Eurytemora hirundoides* on the Swedish coast of the northern Baltic Sea in 1950 was apparently caused by a fungal pathogen, identified later as a new species, *Leptolegnia baltica* (Höhnk and Vallin, 1953). *Eurytemora hirundoides* is the inner Baltic's most common and abundant copepod during the warmer months of the year, constituting 50–90% of all copepods in plankton tows. After the 1950 mortalities the species was much less abundant in samples. Vallin's (1951) description of the mortalities is dramatic:

At the beginning of August 1950 it was reported that the herring fishermen in the Botten Sea, off Sundsvall, had found their nets to be clogged with a sticky substance. When the tackle was taken out of the water the substance soon began to rot and smell. It was suspected that its cause might be industrial pollution from cellulose plants. Samples of the material scraped off the nets, some treated with formalin, were however found to consist of dead plankton crustaceans. A single species of copepod—*Eurytemora hirundoides*—formed almost 100% of the substance. . . . Death of the plankton . . . was first reported from fishing grounds east of the island of Alnö, off Sundsvall, at the beginning of August. Fishermen found such large quantities of dead *Eurytemora* on their nets that they could easily scrape it off by the quart. At the same time the local fishery assistant stated that the sea water in wide areas was made turbid by a lot of "small dead white eggs," which must have been the killed copepods. Within a few days the same phenomenon was reported from other fishing waters. . . . The area, then, stretched for some 70 km in the outer skerries off Sundsvall.

Reports were also received that the mortality was observed on the Finnish coast north of Åland.

Mortalities recurred in 1951, at a reduced level and a month later than in 1950. The severe effects of the disease on *Eurytemora* populations were indicated by reductions in percentages of the species in plankton tows at Sundsvall to only 13%, from an expected 80% characteristic of preepizootic years.

Eurytemora is an important food for herring, *Clupea harengus*, and other pelagic species of the area. Widespread mortalities at the trophic level of copepods could materially reduce the food supply for these commercial fish, in addition to clogging and fouling nets or causing their rapid decay.

Epizootic diseases have been recognized for a long time in the smaller, inconspicuous, and noneconomic Crustacea. Bang (1970) sum-

marized much of the early literature on these diseases, pointing out that when Metchnikoff began his classic studies on the comparative pathology of inflammation (published in 1893), his favorite experimental animal was the water flea *Daphnia*, in which six different types of infections had *already* been described. Others have been described since then.

Bacterial, fungal, and microsporidial infections were described in the amphipod *Gammarus* (a sand flea) 60 years ago (Pixell-Goodrich, 1928), and a hundred years ago a luminescent bacterial disease was described in sand hoppers along the coast of France (Giard and Billet, 1889). A similar disease was later found in sand hoppers (*Talorchestia* and *Orchestia*) along the beaches at Woods Hole, Massachusetts (Inman, 1927).

Other mass mortalities of small crustaceans have been reported. An epizootic caused by the phycomycete *Hyphochytrium peniliae* resulted in a major (98%) population reduction of the cladoceran *Penilia avirostris* in the Black Sea in 1963–1964 (Artemchuk and Zelezinskaya, 1969, cited by Unestam, 1973). Another phycomycete, *Aphanomyces ovidestruens*, was reported to have eliminated some populations of the copepod *Diaptomus gracilis* in central Europe by invading and destroying eggs (Gicklhorn, 1923).

Epizootic diseases in cultured populations of crustaceans are predominantly those of larvae, with a whole new array of facultative pathogens and harmful epibionts which emerge under stress conditions (e.g., poor water quality, inadequate nutrition, and overcrowding primarily). The list of facultative organisms that can kill larval and postlarval crustaceans is growing and is already impressive (Sindermann and Lightner, 1988). Viral and *Vibrio* infections, filamentous bacterial epibionts, ciliate infestations, and fungal (*Lagenidium* and *Sirolopidium*) infections can affect the survival of penaeid shrimp larvae; bacterial necrosis, ciliate infestations, and filamentous bacterial epibionts affect freshwater prawn larvae, while bacterial and fungal (*Lagenidium* and *Haliphthoros*) infections and filamentous bacterial epibionts have been reported in lobster larvae.

This does not imply, of course, that there are no disease problems with cultured juvenile and adult Crustacea. Shell disease seems to be a universal problem, as do *Vibrio* and related infections.

Epizootics in crustacean populations may be classified in one sense as follows:

1. The big "oh-my" outbreaks with accompanying mortalities in natural populations of large commercial species, that command attention and immediate research response

2. The quieter, but probably no less significant, mortalities in populations of the tiny Crustacea—such as copepods, amphipods, and cladocerans
3. The more recent outbreaks of disease in cultured populations—outbreaks that often involve early life history stages, often emerge because of nonoptimum culture conditions, and may affect the viability and profitability of commercial ventures

It would seem reasonable at this point to ask the question: Do crustacean epizootics follow classical epidemiological principles? The typical epidemic wave pattern found in textbooks includes the following phases: prevalence of a pathogen in a susceptible population begins to increase; the number of acute cases and the number of mortalities rise rapidly to a peak; then as the numbers of susceptibles decline and the likelihood of contact between pathogen and susceptible host declines, the wave recedes, in terms of numbers of acute cases and numbers of mortalities. Resistant individuals and chronic infections characterize the declining limb of the curve.

Evidence from crustacean disease outbreaks suggests that

1. An enzootic disease such as gaffkemia in lobsters periodically produces highly significant population losses during outbreaks. So, at least for that disease, there is a strong suggestion of wavelike increases in the abundance of the pathogen in wild populations.
2. With a number of epizootics, a typical pattern emerges. Increasing numbers of infected individuals lead to an epizootic peak, followed by declining levels of infection and declining mortality rates.
3. The crayfish plague in Europe had characteristics of a pandemic of a newly introduced virulent pathogen in a highly susceptible population.
4. Infection pressure seems to play an important role in outbreaks of several diseases.

There is one significant uncertainty, however, in concluding that epizootics in Crustacea follow classical epidemiological principles: that concerns the *nature of the host response to infection*. Several pathogens—*Aerococcus viridans* in lobsters and *Aphanomyces astaci* in European crayfish, for example—fail to elicit effective defensive responses in susceptible hosts. Reassuringly, however, several of the larger Crustacea have, in the last two decades, been found to possess both heightened phagocytic and increased humoral responses to some experimental infections, as reviewed in Chapter 10. If individual resistance is enhanced

by previous exposure to pathogens, it is logical to expect an epizootic wave similar to that seen in vertebrate populations, even if the *mechanisms* of defense are not identical to those of vertebrates.

Several authors have suggested that the regulation of population size by disease may be more common in invertebrates than in vertebrates, primarily because of the absence in invertebrates of specific acquired immune responses to infection (Holmes, 1982; May, 1983). This reasoning seems logical, but discounts or ignores recent evidence for acquired resistance in some invertebrates, which, even though non-specific, may still act to protect the animal from reinfection.

It would also seem reasonable, somewhere in this discussion, to consider in a general way the effects of disease on the abundance of crustacean populations. Mortalities as a consequence of epizootics may range up to 100% of local populations (as is true for crayfish plague), and severe effects on overall abundance may result. Usually, though, the percentages of mortality are lower, and it becomes difficult to separate disease effects from those of other environmental variables. Estimates have been offered on how disease outbreaks can affect populations. Couch (1983), for example, stated that "Any protozoan disease agent that causes losses of 5–10% or greater in crustacean populations may affect natural abundance of a given crustacean species for one or more generations in local areas." Other authors have reported much higher levels of mortalities, but have been reluctant to conclude that any long-term effects on population size resulted.

IV. CONCLUSIONS

A general conclusion seems to be that the internal defense mechanisms of aquatic invertebrates differ from those of vertebrates, in that they lack specificity and depend principally on preformed cellular elements and secreted substances in the circulating body fluid. Furthermore, greater dependence seems to be placed on genetic selection for resistance, rather than on rapid mobilization of defenses by individual animals. These factors lead to the likelihood that the patterns of epizootic waves caused by primary pathogens in invertebrate populations will differ to some extent from those characteristic of vertebrate populations—with a longer time period and often a higher mortality rate extending over many years. The best-documented examples of epizootics in aquatic invertebrates are:

- *Krebspest*, or crayfish plague, in European crayfish, *Astacus astacus*, caused by the phycomycete fungus *Aphanomyces astaci* (1860 to the present)

- Gaffkemia in American and European lobsters, *Homarus americanus* and *Homarus vulgaris*, caused by the micrococcus *A. viridans* var. *homari* (1945 to the present, sporadically)
- Delaware Bay disease of American oysters, *Crassostrea virginica*, caused by the ascetosporan protozoan *Haplosporidium nelsoni* (1957 to the present)
- "Dermo" disease of American oysters, *C. virginica*, caused by the apicomplexan protozoan *Perkinsus marinus* (1944 to the present)

Each of these epizootics is characterized by lengthy periods of high mortalities, reducing the affected populations drastically or eliminating local stocks entirely. As an extreme example, the fungus *A. astaci* has been at what must be considered epizootic levels in European crayfish stocks for 129 years, with no indication of increased resistance in exposed populations or of reduced virulence of the pathogen in remaining susceptible populations on the fringes of the known distribution. On a more local level the protozoan *H. nelsoni* has persisted at epizootic levels in Delaware Bay oysters for more than 30 years—with variable but persistently high annual mortalities and with some indication of slowly developing resistance in each succeeding generation of survivors.

As might be anticipated by their evolutionary distance from one another, molluscs and crustaceans exhibit some differences in individual and population responses to pathogens. The molluscan pattern is represented by high mortalities, with consequent severe genetic selection, over many generations, of individuals resistant to the pathogen—in terms of the containment of invading organisms and a longer survival time of infected hosts. The mechanisms of this resistance are not well understood, but they include activation of phagocytes and the presence of preformed lytic factors in hemolymph and tissues. The crustacean pattern seems to include greater individual responsiveness to foreign invasion, again with an array of hemolymph lytic and agglutinating factors, as well as phagocytic activation, but augmented by some indications of heightened responsiveness after exposure to pathogens. Mortalities in local subpopulations of crustaceans seem to be more circumscribed temporally than is characteristic of molluscan populations, even though repeated outbreaks may occur in both.

One final thought about disease outbreaks in molluscs and crustaceans concerns the origins of epizootics. Disease is clearly an important mechanism of population regulation in invertebrates (Anderson and May, 1981), and the dynamics of its action have been considered by examples in this chapter and in Chapter 10. The question of how epizootics originate in invertebrate populations is, however, still largely unresolved, except by resorting to classical (vertebrate) concepts of an

interplay of host susceptibility, pathogen virulence, and environmental stressors. What may be overlooked is the possible intervention of humans in disrupting a natural dynamic balance by transfers and introductions of virulent pathogens to susceptible stocks through deliberate movements of animals from populations which are resistant to the pathogens, but carry them in latent or chronic states. Evidence for introduced pathogens as a source of major disease outbreaks in molluscs and in crustaceans is highly speculative, but interesting nonetheless. Some examples of coincidental introductions and disease outbreaks in native species are these:

- Mortalities of native oysters in the Gulf of St. Lawrence due to Malpeque Bay disease began in 1915, in an area near where oysters from New England were introduced in 1914.
- Neoplastic disease of soft-shell clams in Chesapeake Bay was first observed in 1978; clams from New England had been transferred to the Bay following hurricane Agnes in 1972, to augment stocks which had suffered an estimated 70% mortality due to low salinities.
- Gill disease (a viral disease) in Portuguese oysters on the French coast was first observed in 1967, 1 year after the first experimental imports of Pacific oysters.
- Delaware Bay disease of oysters (caused by the protozoan *H. nelsoni*) began in a very localized area of Delaware Bay in 1957, and that area has remained an epicenter of the disease.
- European crayfish plague began in 1860 and has since spread throughout much of the subcontinent. The strong suspicion that the disease was imported with shipments of crayfish from the United States is supported by the fact that all European crayfish species are susceptible, whereas all American species are resistant.

It would be a little extreme to claim that every outbreak can be traceable to an imported pathogen, but it is tempting to hypothesize that many (if not most) of the severe recent disease outbreaks in molluscan and crustacean stocks may have been the consequences of transport of infected but resistant nonindigenous hosts.

REFERENCES

- Alderman, D. J. (1969). Progress report on the gill disease of oysters in the United Kingdom. *Int. Counc. Explor. Sea, Doc. C.M.1969/K:15*, 5 pp.
- Alderman, D. J. (1974). Foreign diseases hazard British shellfish. *New Sci.* **63**, 122–124.

- Alderman, D. J., and Gras, P. (1969). Gill disease of Portuguese oysters. *Nature (London)* **224**, 616–617.
- Alderman, D. J., and Jones, E. B. G. (1967). Shell disease of *Ostrea edulis* L. *Nature (London)* **216**, 797–798.
- Alderman, D. J., and Jones, E. B. G. (1971). Shell disease of oysters. *Fish. Invest. Minist. Agric. Fish. Food, G.B.* **26**(8), 14 pp.
- Alderman, D. J., Polglase, J. L., Frayling, M., and Hogger, J. (1984). Crayfish plague in Britain. *J. Fish Dis.* **7**, 401–405.
- Anderson, R. M., and May, R. M. (1981). The population dynamics of microparasites and their invertebrate hosts. *Philos. Trans. R. Soc. London, Ser. B* **291**, 451–524.
- Andrews, J. D. (1964). Oyster mortality studies in Virginia. IV. MSX in James River public seed beds. *Proc. Natl. Shellfish. Assoc.* **53**, 65–84.
- Andrews, J. D. (1965). Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. *Chesapeake Sci.* **6**, 60–67.
- Andrews, J. D. (1966). Oyster mortality studies in Virginia. V. Epizootiology of MSX, protistan pathogen of oysters. *Ecology* **47**, 19–31.
- Andrews, J. D. (1968). Oyster mortality studies in Virginia. VII. Review of epizootiology and origin of *Minchinia nelsoni*. *Proc. Natl. Shellfish. Assoc.* **58**, 23–36.
- Andrews, J. D. (1984). Epizootiology of haplosporidan diseases affecting oysters. *Comp. Pathobiol.* **7**, 243–269.
- Andrews, J. D., and Castagna, M. (1978). Epizootiology of *Minchinia costalis* in susceptible oysters in seaside bays of Virginia's eastern shore 1959–1976. *J. Invertebr. Pathol.* **32**, 124–138.
- Andrews, J. D., and Hewatt, W. G. (1957). Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. *Ecol. Monogr.* **27**, 1–26.
- Andrews, J. D., and Wood, J. L. (1967). Oyster mortality studies in Virginia. VI. History and distribution of *Minchinia nelsoni*, a pathogen of oysters, in Virginia. *Chesapeake Sci.* **8**, 1–13.
- Andrews, J. D., Wood, J. L., and Hoese, H. D. (1962). Oyster mortality studies in Virginia. III. Epizootiology of a disease caused by *Haplosporidium costale* Wood and Andrews. *J. Insect Pathol.* **4**, 327–343.
- Artemchuk, N. Y., and Zelezinskaya, L. M. (1969). The marine fungus, *Hyphochytrium peniliae* n. sp., affecting the zooplankton crustacean *Penilia avirostris* (Dana). *Mikol. Fitopatol.* **3**, 356–358.
- Balouet, G., Poder, M., and Cahour, A. (1983). Haemocytic parasitosis: Morphology and pathology of lesions in the French flat oyster, *Ostrea edulis* L. *Aquaculture* **34**, 1–14.
- Bang, F. B. (1970). Disease mechanisms in crustacean and marine arthropods. *Am. Fish. Soc., Spec. Publ.* **5**, 383–404.
- Beattie, J. H., Chew, K. K., and Hershberger, W. K. (1980). Differential survival of selected strains of Pacific oysters (*Crassostrea gigas*) during summer mortality. *Proc. Natl. Shellfish. Assoc.* **70**, 184–189.
- Benisch, J. (1940). Künstlich hervorgerufener *Aphanomyces*-Befall bei Wollhandskrabben. *Z. Fisch. Deren Hilfswiss.* **38**, 71–80.
- Bonami, J. -R. (1977). Les maladies virales des crustacés et des mollusques. *Oceanis* **3**, 154–175.
- Bonami, J. -R., Grizel, H., Vago, C., and Duthoit, J. -L. (1971). Recherches sur une maladie épzootique de l'huître plate *Ostrea edulis* Linné. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **35**, 415–418.
- Brongersma-Sanders, M. (1957). Mass mortality in the sea. In "Treatise on Marine Ecology

- and Paleocology: Vol. I. Ecology" (J. Hedgpeth, ed.), pp. 941–1010. Geol. Soc. Am., New York.
- Cerruti, A. (1941). Osservazioni ed esperimenti sulle cause di distruzione delle larve d'ostrea nel Mar Piccola e nel Mar Grande di Taranto. *Arch. Oceanogr. Limnol.* **1**, 165–201.
- Chun, S. K. (1970). Studies on the oyster diseases. 1. Pathogenetic investigation. *Bull. Korean Fish. Soc.* **3**(1), 7–18.
- Cole, H. A. (1950). Shell disease in re-laid French oysters. *Nature (London)* **166**, 19–20.
- Cole, H. A. (1951). The British oyster industry and its problems. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **2**, 7–17.
- Cole, H. A., and Hancock, D. A. (1956). Progress in oyster research in Britain 1949–54, with special reference to the control of pests and diseases. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **3**, 24–29.
- Cole, H. A., and Waugh, G. C. (1956). Shell disease in Portuguese oysters. *Nature (London)* **178**, 422.
- Comps, M. (1969). Observations relatives à l'affection branchiale des huîtres portugaises (*Crassostrea angulata* Lmk.). *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **33**(2), 150–151.
- Comps, M. (1970). Observations sur les causes d'une mortalité anormale des huîtres plates dans le Bassin de Marennes. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **34**, 317–326.
- Comps, M. (1978). Évolution des recherches et études récentes en pathologie des huîtres. *Oceanol. Acta* **1**, 255–262.
- Comps, M., and Duthoit, J.-L. (1976). Infection virale associée à la "maladie des branchies" de l'huître portugaise *Crassostrea angulata* Lmk. *C. R. Hebd. Séances Acad. Sci., Ser. D* **283**, 1595–1596.
- Comps, M., Bonami, J.-R., Vago, C., and Campillo, A. (1976). Une virose de l'huître portugaise (*Crassostrea angulata* Lmk.). *C. R. Hebd. Séances Acad. Sci., Ser. D* **282**, 1991–1993.
- Comps, M., Tige, G., and Grizel, H. (1980). Étude ultrastructurale d'un protiste parasite de l'huître *Ostrea edulis* L. *C. R. Hebd. Séances Acad. Sci., Ser. D* **290**, 383–384.
- Couch, J. A. (1967). Concurrent haplosporidian infections of the oyster, *Crassostrea virginica* (Gmelin). *J. Parasitol.* **53**, 248–253.
- Couch, J. A. (1983). Diseases caused by Protozoa. In "The Biology of Crustacea," Vol. 6, "Pathobiology" (A. J. Provenzano, Jr., ed.), pp. 79–111. Academic Press, New York.
- Couch, J. A., and Rosenfield, A. (1968). Epizootiology of *Minchinia costalis* and *Minchinia nelsoni* in oysters introduced into Chincoteague Bay, Virginia. *Proc. Natl. Shellfish. Assoc.* **58**, 51–59.
- Cukerzis, Y. M. (1964). K voprosu o chum rakov. *Tr. Akad. Nauk Lit. SSR, Ser. B* **1.33**, 77–85.
- Davis, H. C., Loosanoff, V. L., Weston, W. H., and Martin, C. (1954). A fungus disease in clam and oyster larvae. *Science* **120**, 36–38.
- Deltreil, J. -P. (1969a). Deux cas d'affection massive par la maladie des branchies observés sur les huîtres portugaises, *Crassostrea angulata* Lmk., élevées en suspension dans le Bassin d'Arcachon. *Int. Counc. Explor. Sea, Doc.* **C.M.1969/K:7**, 5 pp.
- Deltreil, J. -P. (1969b). Remarques sur la croissance en élevage suspendu de *Crassostrea angulata* Lmk. affectée par la maladie des branchies dans le Bassin d'Arcachon. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **33**, 176–180.
- Deltreil, J. -P. (1971). Étude comparée du comportement de trois lots d'huîtres portugaises *Crassostrea angulata* Lmk., de différentes origines en relation avec l'évolution de la maladie des branchies dans le Bassin d'Arcachon. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **35**, 89–97.

- Deltreil, J. -P. (1973). Évolution et conséquences de la mortalité de *C. angulata* dans le Bassin d'Arcachon et l'estuaire de la Gironde de 1971 à 1973. *Int. Counc. Explor. Sea, Doc. C.M.1973/K:14*, 6 pp.
- Dickie, L. M., and Medcof, J. C. (1963). Causes of mass mortalities of scallops (*Placopecten magellanicus*) in the southwestern Gulf of Saint Lawrence. *J. Fish. Res. Board Can.* **20**, 451-482.
- Dollfus, R. P. (1921). Résumé de nos principales connaissances pratiques sur les maladies et les ennemis de l'huître. *Notes. Mem. Off. Sci. Tech. Pêches Marit.* **7**, 1-46.
- Dollfus, R. P. (1923). La maladie des moules et la mortalité des huîtres en Zélande au cours de ces dernières années. *Bull. Soc. Cent. Aquicult. Pêche* **30**, 38-44.
- Drinnan, R. E., and England, L. A. (1965). Further progress in rehabilitating oyster stocks. *Fish. Res. Board Can. Biol. Stn., St. Andrews, Gen. Ser. Circ.* **48**, 1-4.
- Elston, R. A., Farley, C. A., and Kent, M. L. (1986). Occurrence and significance of bonaimiasis in European flat oysters *Ostrea edulis* in North America. *Dis. Aquat. Org.* **2**, 49-54.
- Engle, J. B., and Rosenfield, A. (1963). Progress in oyster mortality studies. *Proc. Gulf Caribb. Fish. Inst.* **15**, 116-124.
- Eyre, J. W. H. (1923). Some notes on the bacteriology of the oyster (including description of two new species). *J. R. Microsc. Soc.* **1923**, 385-394.
- Eyre, J. W. H. (1924). An account of investigations into the cause or causes of the unusual mortality among oysters in English oyster beds during 1920 and 1921. Part 2, Appendix D. Final bacteriological report. *Fish. Invest. Minist. Agric. Fish. Food, G.B.* **4**, 29-39.
- Farley, C. A. (1968). *Minchinia nelsoni* (Haplosporida, Haplosporidiidae) disease syndrome in the American oyster, *Crassostrea virginica*. *J. Protozool.* **15**, 585-599.
- Farley, C. A. (1989). Selected aspects of neoplastic progression in molluscs. In "Cancer Growth and Progression: Vol. 5. Comparative Aspects of Tumor Development" (H. E. Kaiser, ed.), pp. 24-31. Kluwer Academic, New York.
- Farley, C. A., Otto, S. V., and Reinisch, C. L. (1986). New occurrence of epizootic sarcoma in Chesapeake Bay soft shell clams (*Mya arenaria*). *Fish. Bull.* **84**, 851-857.
- Fischer, P. H. (1951). Causes de destruction des mollusques. Maladies et mort. *J. Conchyliol.* **91**, 29-59.
- Ford, S. E., and Haskin, H. H. (1982). History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957-1980. *J. Invertebr. Pathol.* **40**, 118-141.
- Fujita, T., Matsubara, T., Hirokawa, H., and Araki, F. (1953). On the inflammatorious changes of the *Ostrea gigas* in Hiroshima Bay. *Nippon Suisan Gakkaishi* **19**, 766-770 (in Japanese; English summary).
- Fujita, T., Matsubara, T., Hirokawa, H., and Araki, F. (1955). On the inflammatorious changes of the *Ostrea gigas* in Hiroshima Bay. 2. *Nippon Suisan Gakkaishi* **20**, 1063-1065 (in Japanese; English summary).
- Gaarder, T., and Alvsaker, E. (1951). Biologie und Chemie der Auster in den norwegischen Pollen. *Bergens Mus. Arbok, Naturvitensk. Rekke* **6**, 1-236.
- Giard, A. (1894). Sur une affection parasitaire de l'huître (*Ostrea edulis* L.) connue sous le nom de "maladie du pied." *C. R. Soc. Biol., Paris* **46**, 401-403.
- Giard, A., and Billet, A. (1889). Observations sur la maladie phosphorescente des talitres et autre crustacés. *C. R. Soc. Biol.* **41**, 593-597.
- Gicklhorn, J. (1923). *Aphanomyces ovidestruens* nov. spec., ein Parasit in den Eiern von *Diaptomus*. *Lotos* **71**, 143-156.
- Glude, J. B. (1975). A summary report of Pacific coast oyster mortality investigations 1965-72. *Proc. U.S.-Jpn. Meet. Aquacult.*, 3rd pp. 1-28.

- Goggins, P. L., and Hurst, J. W., Jr. 1960. Progress report on lobster gaffkyaremia (red tail). *Maine Dep. Sea Shore Fish.* 9 pp. (mimeo).
- Grizel, H., and Tige, G. (1973). La maladie de la glande digestive d'*Ostrea edulis* Linné. *Int. Counc. Explor. Sea, Doc. C.M.1973/K:13*, 7 pp.
- Grizel, H., Comps, M., Cousserans, F., Bonami, J.-R., and Vago, C. (1974a). Étude d'un parasite de la glande digestive observé au cours de l'épizootie actuelle de l'huître plate. *C. R. Acad. Sci., Ser. D* **279**, 783–784.
- Grizel, H., Comps, M., Bonami, J.-R., Cousserans, F., Duthoit, J. -L., and LePennec, M. A. (1974b). Recherche sur l'agent de la maladie de la glande digestive de *Ostrea edulis* Linné. *Sci. Pêche* **240**, 7–30.
- Gross, F., and Smyth, J. C. (1946). The decline of oyster populations. *Nature (London)* **157**, 540–542.
- Gulka, G., Chang, P. W., and Marti, K. A. (1983). Prokaryotic infection associated with a mass mortality of the sea scallop, *Placopecten magellanicus*. *J. Fish Dis.* **6**, 355–364.
- Gutiérrez, M., and Pascual, E. (1975). Note préliminaire sur une maladie de la glande digestive de *Crassostrea angulata* (Lmk.). *Int. Counc. Explor. Sea, Doc. C.M.1975/K:6*, 3 pp.
- Haskin, H. H. (1961). Delaware Bay oyster mortalities. *Proc. Gulf. Caribb. Fish. Inst.* **13**, 109 (abstract).
- Haskin, H. H., Stauber, L. A., and Mackin, J. G. (1966). *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae) causative agent of the Delaware Bay oyster epizootic. *Science* **153**, 1414–1416.
- Herrbach, B. (1971). Sur une affection parasitaire de la glande digestive de l'huître plate, *Ostrea edulis* Linné. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **35**, 79–87.
- Herrick, F. H. (1909). Natural history of the American lobster. *Bull. U.S. Bur. Fish.* **29**, 149–408.
- Hershberger, W. K., Perdue, J. A., and Beattie, J. H. (1984). Genetic selection and systematic breeding in Pacific oyster culture. *Aquaculture* **39**, 237–245.
- Hewatt, W. G., and Andrews, J. D. (1956). Temperature control experiments on the fungus disease, *Dermocystidium marinum*, of oysters. *Proc. Natl. Shellfish. Assoc.* **46**, 129–133.
- Hickey, C. R., Jr. (1978). Investigation and analysis of a mass mortality of commercial lobsters (*Homarus americanus*) in Seabrook Harbor, New Hampshire, during October of 1977. *U.S. Nucl. Regul. Comm. [Rep.] NUREG 0468*, 93 pp.
- Hirsch, C. A. E. (1921). Ueber eine Muschelkrankheit in Holland. *Fischerbote* **13**, 217–223.
- Hoese, H. D. (1964). Studies on oyster scavengers and their relation to the fungus *Dermocystidium marinum*. *Proc. Natl. Shellfish. Assoc.* **53**, 161–174.
- Höhnk, W., and Vallin, S. (1953). Epidemisches Absterben von *Eurytemora* im Bottnischen Meerbusen, verursacht durch *Leptolegnia baltica* nov. spec. *Veroff. Inst. Meeresforsch. Bremerhaven* **2**, 215–223.
- Holmes, J. C. (1982). Impact of infectious disease agents on the population growth and geographical distribution of animals. In "Population Biology of Infectious Diseases" (R. M. Anderson and R. M. May, eds.), pp. 37–51. Springer-Verlag, Berlin.
- Hopkins, S. H. (1957). Interrelations of organisms. B. Parasitism. In "Treatise on Marine Ecology and Paleoecology: Vol. I. Ecology" (J. W. Hedgpeth, ed.), pp. 413–428. Geol. Soc. Am., New York.
- Hornell, J. (1910). The practice of oyster culture at Arcachon and its lessons for India. *Madras Fish. Bull.* **2**(5), 1–90.
- Howell, B., Key, D., and Shotton, R. (1968). Interim report on investigations into gill diseases of Portuguese oysters. *Int. Counc. Explor. Sea, Doc. C.M.1968/K:12*, 8 pp.
- Imai, T., Numachi, L., Oizumi, J., and Sato, S. (1965). Studies on the mass mortality of the

- oyster in Matsushima Bay. 2. Search for the cause of mass mortality and the possibility to prevent it by transplantation experiment. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 27–38.
- Ingalls, R. A., and Needler, A. W. H. (1939). Preliminary report on shore mollusc resources of the Northumberland Strait coast of Nova Scotia. *Fish. Res. Board Can., Ms. Rep. Biol. Stn.* **161** 1–26.
- Ingalls, R. A., and Needler, A. W. H. (1940). A report on shore mollusc resources of the Northumberland Strait coast of Nova Scotia. *Fish. Res. Board Can., Ms. Rep. Biol. Stn.* **333**, 1–29.
- Inman, O. L. (1927). A pathogenic luminescent bacterium. *Biol. Bull. (Woods Hole, Mass.)* **53**, 197–200.
- Jepps, M. J. (1937). On the protozoan parasites of *Calanus finmarchicus* in the Clyde Sea area. *J. Microsc. Sci.* **79**, 589–658.
- Johnson, P. T. (1977). Paramoebiasis in the blue crab, *Callinectes sapidus*. *J. Invertebr. Pathol.* **29**, 308–320.
- Kan-no, H., Sasaki, M., Sakurai, Y., Watanabe, T., and Suzuki, K. (1965). Studies on the mass mortality of the oyster in Matsushima Bay. 1. General aspects of the mass mortality of the oyster in Matsushima Bay and its environmental conditions. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 1–26.
- Katkansky, S. C., and Warner, R. W. (1974). Pacific oyster disease and mortality studies in California. *Mar. Resour. Tech. Rep., Calif. Dep. Fish Game* **25**, 51 pp.
- Kern, F. G., Sullivan, L. C., and Takata, M. (1973). *Labyrinthomyxa*-like organisms associated with mass mortalities of oysters, *Crassostrea virginica*, from Hawaii. *Proc. Natl. Shellfish. Assoc.* **63**, 43–46.
- Koganezawa, A. (1975). Present status of studies on the mass mortality of cultured oysters in Japan and its prevention. *Proc. U.S.-Jpn. Meet. Aquacult.*, 3rd pp. 29–34.
- Korringa, P. (1947). Les vicissitudes de l'ostréiculture hollandaise élucidées par la science ostréicole moderne. *Ostréicult. Cult. Mar.* **16**, 3–9.
- Korringa, P. (1951a). Investigations on shell disease in the oyster, *Ostrea edulis* L. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **128**(2), 50–54.
- Korringa, P. (1951b). On the nature and function of "chalky" deposits in the shell of *Ostrea edulis* Linnaeus. *Proc. Calif. Acad. Sci.* **27**(5), 133–158.
- Korringa, P. (1952). Recent advances in oyster biology. *Q. Rev. Biol.* **27**, 266–308 and 339–365.
- Kozłowski, B. (1968). Dżuma raków w woj. kieleckim. *Med. Weter.* **24**, 353–355.
- Leuckart, R. (1884). Die Ursachen der Krebspest. *Circ. Dtsch. Fisch.-Vereins* 1884 pp. 62–65.
- Li, M. F., and Clyburne, S. (1979). Mortalities of blue mussel (*Mytilus edulis*) in Prince Edward Island. *J. Invertebr. Pathol.* **33**, 108–110.
- Lipovsky, V. P., and Chew, K. K. (1972). Mortality of Pacific oysters (*Crassostrea gigas*): The influence of temperature and enriched seawater on survival. *Proc. Natl. Shellfish. Assoc.* **62**, 72–82.
- Logie, R. R. (1956). Oyster mortalities, old and new, in the Maritimes. *Fish Res. Board Can., Prog. Rep. Atl. Coast. Stn.* **65**, 3–11.
- Logie, R. R. (1958a). Epidemic oyster disease and rehabilitation transfers in 1957. *Fish. Res. Board Can., Gen. Ser. Circ.* **31**, 2 pp.
- Logie, R. R. (1958b). "Epidemic Disease in Canadian Atlantic Oysters," Ph.D. thesis, Rutgers University (Univ. Microfilms), 143 pp. New Brunswick, New Jersey (also issued as *Fish Res. Board Can., Ms. Rep. Biol. Stn.* **661**, 91 pp.).
- Logie, R. R., Drinnan, R. E., and Henderson, E. B. (1960). Rehabilitation of disease-depleted oyster populations in eastern Canada. *Proc. Gulf Caribb. Fish. Inst.* **13**, 109–113.

- Mackin, J. G. (1960). Status of researches on oyster disease in North America. *Proc. Gulf Caribb. Fish. Inst.* **13**, 98–113.
- Mackin, J. G. (1961). Mortalities of oysters. *Proc. Natl. Shellfish. Assoc.* **51**, 21–40.
- Mackin, J. G. (1962). Oyster disease caused by *Dermocystidium marinum* and other micro-organisms in Louisiana. *Publ. Inst. Mar. Sci.* **7**, 132–229.
- Mackin, J. G., Owen, H. M., and Collier, A. (1950). Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). *Science* **111**, 328–329.
- Mannsfeld, W. (1942). Die Krebspest im Generalbezirk Lettland in den Jahren 1924–1938. *Z. Fisch. Deren Hilfswiss* **40**, 395–417.
- Marteil, L. (1968). La "maladie des branchies." *Int. Counc. Explor. Sea, Doc.* **C.M.1968/K:5**, 5 pp.
- Marteil, L. (1969). Données générales sur la maladie des branchies. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **33**, 145–150.
- Marteil, L. (1976). La conchyliculture française. 2^e partie. Biologie de l'huître et de la moule. *Rev. Trav. Inst. Sci. Tech. Pêches Marit.* **40**, 149–345.
- May, R. M. (1983). Parasitic infections as regulators of animal populations. *Am. Sci.* **71**, 36–45.
- Medcof, J. C., and Bourne, N. (1964). Causes of mortality of the sea scallop, *Placopecten magellanicus*. *Proc. Natl. Shellfish. Assoc.* **53**, 33–50.
- Merrill, A. S., and Posgay, J. A. (1964). Estimating the natural mortality rate of the sea scallop (*Placopecten magellanicus*). *Int. Comm. N.W. Atl. Fish., Res. Bull.* **1**, 88–106.
- Mori, K. (1967). Histochemical study on the localization and physiological significance of glucose-6-phosphate dehydrogenase system in the oyster during the stages of sexual maturation and spawning. *Tohoku J. Agric. Res.* **17**(4), 287–301.
- Mori, K. (1968). Changes of oxygen consumption and respiratory quotient in the tissues of oysters during the stages of sexual maturation and spawning. *Tohoku J. Agric. Res.* **19**(2), 136–143.
- Mori, K., Imai, T., Toyoshima, K., and Usuki, I. (1965a). Studies on the mass mortality of the oyster in Matsushima Bay. 4. Changes in the physiological activity and the glycogen content of the oyster during the stages of sexual maturation and spawning. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 49–63.
- Mori, K., Tamate, H., Imai, T., and Itikawa, O. (1965b). Studies on the mass mortality of the oyster in Matsushima Bay. 5. Changes in the metabolism of lipids and glycogen of the oyster during the stages of sexual maturation and spawning. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 65–88.
- Mori, K., Tamate, H., and Imai, T. (1966) Histochemical study on the change of 17 β -hydroxysteroid dehydrogenase activity in the oyster during the stages of sexual maturation and spawning. *Tohoku J. Agric. Res.* **17**(2), 179–191.
- Needler, A. W. H., and Logie, R. R. (1947). Serious mortalities in Prince Edward Island oysters caused by a contagious disease. *Trans. R. Soc. Can., Sect. 5* **41**, 73–89.
- Newman, M., and Ward, G. E., Jr. (1973). An epizootic of blue crabs, *Callinectes sapidus*, caused by *Paramoeba perniciosus*. *J. Invertebr. Pathol.* **22**, 329–334.
- Nikolić, M. (1964). Causes of oyster mass mortality, Limski Kanal, Istra, 1960. *Acta Adriat.* **11**(31), 227–238.
- Ninni, A. P. (1865). Sulla mortalità dei gambari (*Astacus fluviatilis* L.) nel veneto e più particolarmente nella provincia trevigiana. *Atti Ist. Veneto, Ser. III* **10**, 1203–1209.
- Numachi, K., Oizumi, J., Sato, S., and Imai, T. (1965). Studies on the mass mortality of the oyster in Matsushima Bay. 3. The pathological degenerations of oysters by gram-positive bacteria and their frequency. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 39–47.

- Nybelin, O. (1935). Über die Ursache der Krebspest in Schweden. *Fischereizeitung* **38**, 21.
- Nybelin, O. (1936). Untersuchungen über die Ursache der in Schweden gegenwärtig vorkommenden Krebspest. *Rep. Inst. Freshwater Res., Drottningholm* **9**, 3–29.
- Ogasawara, Y., Kobayashi, U., Okamoto, R., Furukawa, A., Hisaoka, M., and Nogami, K. (1962) The use of suppressed oyster seed spats in the oyster culture and its productive significance. *Nankei-ku Suisan Kenkyusho Hokoku* **19**, 1–153.
- Orton, J. H. (1924a). An account of the investigations into the cause or causes of the unusual mortality among oysters in English oyster beds during 1920 and 1921. Part I. *Fish. Invest. Minist. Agric. Fish. Food, G.B.* **6**(3), 1–99.
- Orton, J. H. (1924b). An account of the investigations into the cause or causes of the unusual mortality among oysters in English oyster beds during 1920 and 1921. Part 2. Appendix A. Interim report on oyster mortality investigations, 1920. *Fish. Invest. Minist. Agric. Fish. Food, G.B.* **6**(4), 3–14.
- Orton, J. H. (1937). "Oyster Biology and Oyster Culture. Buckland Lectures for 1935." London, Arnold.
- Perdue, J. A., Beattie, J. H., and Chew, K. K. (1981). Some relationships between gametogenic cycle and summer mortality phenomenon in the Pacific oyster (*Crassostrea gigas*). *J. Shellfish Res.* **1**, 9–16.
- Pereyra, W. T. (1964). Mortality of Pacific oysters, *Crassostrea gigas* (Thunberg), in various exposure situations in Washington. *Proc. Natl. Shellfish. Assoc.* **53**, 51–63.
- Perkins, F. O. (1976). *Dermocystidium marinum* infection in oysters. *Mar. Fish. Rev.* **38**(10), 19–21.
- Perkins, F. O., and Wolf, P. H. (1976). Fine structure of *Marteilia sydneyi* sp. n.—haplosporidan pathogen of Australian oysters. *J. Parasitol.* **62**, 528–538.
- Pixell-Goodrich, H. L. (1928). Reactions of *Gammarus* to injury and disease with notes on microsporidial and fungoid diseases. *Q. J. Microsc. Sci.* **72**, 325–353.
- Pixell-Goodrich, H. L. (1956). Crayfish epidemics. *Parasitology* **46**, 480–483.
- Poder, M., Cahour, A., and Balouet, G. (1982). Hemocytic parasitosis in European oyster *Ostrea edulis* L.: Pathology and contamination. *Proc. Int. Colloq. Invertebr. Pathol.*, 3rd pp. 254–257.
- Quick, J. A., and Mackin, J. G. (1971). Oyster parasitism by *Labyrinthomyxa marina* in Florida. *Fla., Dep. Nat. Resour., Prof. Pap. Ser.* **13**, 55 pp.
- Ray, S. M. (1952). A culture technique for diagnosis of infections with *Dermocystidium marinum* Mackin, Owen and Collier in oysters. *Science* **116**, 360–361.
- Ray, S. M. (1954a). Biological studies of *Dermocystidium marinum*, a fungus parasite of oysters. *Pam. Rice Inst. (Monogr. Biol.)*, Spec. Issue, 114 pp.
- Ray, S. M. (1954b). Experimental studies on the transmission and pathogenicity of *Dermocystidium marinum*, a fungus disease of oysters. *J. Parasitol.* **40**, 235.
- Ray, S. M. (1966a). Notes on the occurrence of *Dermocystidium marinum* on the Gulf of Mexico coast during 1961 and 1962. *Proc. Natl. Shellfish. Assoc.* **54**, 45–54.
- Ray, S. M. (1966b). A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proc. Natl. Shellfish. Assoc.* **54**, 55–69.
- Ray, S. M., and Chandler, A. C. (1955). *Dermocystidium marinum*, a parasite of oysters. *Exp. Parasitol.* **4**, 172–200.
- Ray, S. M., Mackin, J. G., and Boswell, J. L. (1953). Quantitative measurement of the effect on oysters of disease caused by *Dermocystidium marinum*. *Bull. Mar. Sci.* **3**, 6–33.
- Reinhard, E. G. (1956). Parasitic castration of Crustacea. *Exp. Parasitol.* **5**, 79–107.
- Rennerfelt, E. (1936). Untersuchungen über die Entwicklung und Biologie des Krebspestpilzes *Aphanomyces astaci* Schikora. *Rep. Inst. Freshwater Res., Drottningholm* **10**, 1–21.

- Rosenfield, A. (1976). Environmental studies of neoplasms in marine shellfish. *Prog. Exp. Tumor Res.* 20, 203–212.
- Rosenfield, A., and Sindermann, C. J. (1966). The distribution of "MSX" in middle Chesapeake Bay. *Proc. Natl. Shellfish. Assoc.* 56, 6 (abstract).
- Roskam, R. T. (1957). Gaffkaemia, a contagious disease in *Homarus vulgaris*. *Int. Counc. Explor. Sea, Shellfish Comm. Rep.* 1957/1, 1–4.
- Roughley, T. C. (1926). An investigation of the cause of an oyster mortality on the George's River, New South Wales, 1924–25. *Proc. Linn. Soc. N.S.W.* 51, 446–491.
- Sanders, M. J. (1966). Victorian offshore scallop explorations. *Aust. Fish. Newsl.* 25, 11 and 13.
- Sawyer, T. K. (1969). Preliminary study on the epizootiology and host–parasite relationship of *Paramoeba* sp. in the blue crab, *Callinectes sapidus*. *Proc. Natl. Shellfish. Assoc.* 59, 60–64.
- Schäperclaus, W. (1935). Die Ursache der pestartigen Krebssterben. *Z. Fisch.* 33, 343–366.
- Schikora, F. (1903). Über die Krebspest und ihren Erreger. *Fischereizeitung* 6, 353–355.
- Schikora, F. (1906). Die Krebspest. *Fischereizeitung* 9, 529–532, 549–553, 561–566, and 581–583.
- Schikora, F. (1916). "Die Wiederbevölkerung der Deutschen Gewässer mit Krebsen," 195 pp. Neumann, Neudamm, Germany.
- Schikora, F. (1926). 50 Jahre Krebspest. *Fischereizeitung* 29, 225–228 and 250–253.
- Seligo, A. (1895). Bemerkungen über Krebspest Wasserpest, Lebensverhältnisse des Krebses. *Z. Fisch. Deren Hilfswiss.* 3, 247–261.
- Sindermann, C. J. (1965). Effect of environment on several diseases of herring from the western North Atlantic. *Int. Comm. N.W. Atl. Fish., Spec. Publ.* 6, 603–610.
- Sindermann, C. J. (1968a). Oyster mortalities, with particular reference to Chesapeake Bay and the Atlantic coast of North America *U.S. Fish Wildl. Serv., Spec. Sci. Rep.—Fish.* 569, 10 pp.
- Sindermann, C. J. (1968b). Bibliography of oyster parasites and diseases. *U.S. Fish Wildl. Serv., Spec. Sci. Rep.—Fish.* 563, 13 pp.
- Sindermann, C. J. (1970). "Principal Diseases of Marine Fish and Shellfish," 369 pp. Academic Press, New York.
- Sindermann, C. J. (1975). Status of disease and mortality investigations of the Pacific oyster in 1975. A report to the UJNR Aquaculture Panel. *Natl. Mar. Fish. Serv., Inf. Rep. Middle Atl. Coast. Fish. Cent.* 83, 11 pp.
- Sindermann, C. J. (1976). Oyster mortalities and their control. *FAO Tech. Conf. Aquacult.* 76/R34, 25 pp.
- Sindermann, C. J., and Farrin, A. E. (1962). Ecological studies of *Cryptocotyle lingua* (Trematoda: Heterophyidae) whose larvae cause "pigment spots" of marine fish. *Ecology* 43, 69–75.
- Sindermann, C. J., and Lightner, D. V. (Eds.) (1988). "Disease Diagnosis and Control in North American Marine Aquaculture," 431 pp. Elsevier, New York.
- Sindermann, C. J., and Rosenfield, A. (1967). Principal diseases of commercially important marine bivalve Mollusca and Crustacea. *U.S. Fish Wildl. Serv., Fish. Bull.* 66, 335–385.
- Spärrck, R. (1950). Investigation on the biology of the oyster. 12. On the fluctuations in the oyster stock of northwestern Europe. *Rep. Dan. Biol. Stn.* 52, 41–50.
- Sparks, A. K. (1985). "Synopsis of Invertebrate Pathology Exclusive of Insects," 423 pp. Elsevier, Amsterdam.
- Sprague, V. (1963). Revision of genus *Haplosporidium* and restoration of genus *Minchinia* (Haplosporida, Haplosporidiidae). *J. Protozool.* 10, 263–266.
- Sprague, V. (1971). Diseases of oysters. *Annu. Rev. Microbiol.* 25, 211–230.

- Sprague, V., and Beckett, R. L. (1966). A disease of blue crabs (*Callinectes sapidus*) in Maryland and Virginia. *J. Invertebr. Pathol.* **8**, 287–289.
- Sprague, V., and Beckett, R. L. (1968). The nature of the etiological agent of “gray crab” disease. *J. Invertebr. Pathol.* **11**, 503.
- Sprague, V., Beckett, R. L., and Sawyer, T. K. (1969). A new species of *Paramoeba* (Amoebida, Paramoebidae) parasitic in the crab *Callinectes sapidus*. *J. Invertebr. Pathol.* **14**, 167–174.
- Stewart, J. E. (1984). Lobster diseases. *Helgol. Wiss. Meeresunters.* **37**, 243–254.
- Stewart, J. E., Cornick, J. W., Spears, D. I., and McLeese, D. W. (1966). Incidence of *Gaffkya homari* in natural lobster (*Homarus americanus*) populations of the Atlantic region of Canada. *J. Fish. Res. Board Can.* **23**, 1325–1330.
- Takeuchi, T. (1963). Study of measures to control oyster mortalities. *Hiroshima-ken Suisan Shikenjo Jikyo Hokoku* **24**, 29–46 (in Japanese).
- Takeuchi, T., Matsubara, T., Hirokawa, Y., and Tsukiyama, A. (1955). Bacteriological studies on the unusually high mortality of *Ostrea gigas* in Hiroshima Bay. 1. *Nippon Suisan Gakkaishi* **20**, 1066–1070.
- Takeuchi, T., Matsubara, T., Hirokawa, Y., and Tsukiyama, A. (1956). Bacteriological studies on the unusually high mortality of *Ostrea gigas* in Hiroshima Bay. 2. *Nippon Suisan Gakkaishi* **21**, 1199–1203.
- Takeuchi, T., Matsubara, T., Hirokawa, Y., and Matsuo, Y. (1957). Bacteriological studies on the unusually high mortality of *Ostrea gigas* in Hiroshima Bay. 3. *Nippon Suisan Gakkaishi* **23**, 19–23.
- Takeuchi, T., Takemoto, Y., and Matsubara, T. (1960). Haematological study of bacteria affected oysters. *Rep. Hiroshima Pref. Fish. Exp. Stn.* **22**(1), 1–7 (in Japanese, translated by the U.S. Joint Publ. Res. Serv. for Transl. Prog., Bur. Comm. Fish., 1965.)
- Tamate, H., Numachi, K., Mori, K., Itikawa, O., and Imai, T. (1965). Studies on the mass mortality of the oyster in Matsushima Bay. 6. Pathological studies. *Tohoku-ku Suisan Kenkyusho Kenkyu Hokoku* **25**, 89–104.
- Tige, G., and Morel, M. (1974). Extension de la maladie de la glande digestive de l’huître plate en Bretagne. *Int. Counc. Explor. Sea, Doc.* **C.M.1974/K:37**, 7 pp.
- Tubiash, H. S., Chanley, P. E., and Leifson, E. (1965). Bacillary necrosis, a disease of larval and juvenile bivalve molluscs. 1. Etiology and epizootiology. *J. Bacteriol.* **90**, 1034–1044.
- Unestam, T. (1964). Kräftpesten. *Fiske* **1964**, 3–7.
- Unestam, T. (1969a). Resistance to the crayfish plague in some American, Japanese, and European crayfishes. *Rep. Inst. Freshwater Res., Drottningholm* **49**, 202–209.
- Unestam, T. (1969b). On the adaptation of *Aphanomyces astaci* as a parasite. *Physiol. Plant.* **22**, 221–235.
- Unestam, T. (1969c). Studies on *Aphanomyces astaci*, physiology and parasitic relationship to the crayfish. *Acta Univ. Ups., Abstr. Uppsala Diss. Fac. Sci.* **134**, 1–22.
- Unestam, T. (1972). On the host range and origin of the crayfish plague fungus. *Rep. Inst. Freshwater Res., Drottningholm* **52**, 192–198.
- Unestam, T. (1973). Fungal diseases of Crustacea. *Rev. Med. Vet. Mycol.* **8**, 1–20.
- Unestam, T., and Weiss, D. W. (1970). The host-parasite relationship between freshwater crayfish and the crayfish disease fungus *Aphanomyces astaci*: Responses to infection by a susceptible and a resistant species. *J. Gen. Microbiol.* **60**, 77–90.
- Vallin, S. (1951). Plankton mortality in the northern Baltic caused by a parasitic water-mould. *Rep. Inst. Freshwater Res., Drottningholm* **32**, 139–148.
- Vilela, H. (1968). L’état actuel des huïtieries et des huîtres en Portugal. *Int. Counc. Explor. Sea, Doc.* **C.M.1968/K:17**, 4 pp.
- Vilela, H., and Sampayo, M. A. (1969). La maladie des branchies chez les huîtres et les moules. *Int. Counc. Explor. Sea, Doc.* **C.M.1969/K:13**, 5 pp.

- Voisin, P. (1931). Biologie ostréicole. La maladie des huîtres de Zélande. *Rev. Trav. Off. Sci. Tech. Pêches Marit.* **4**, 221–222.
- Walne, P. R. (1961). Observations on the mortality of *Ostrea edulis*. *J. Mar. Biol. Assoc. U.K.* **41**, 113–122.
- Weber, A. (1899). Zur Aetiologie der Krebspest. *Arb. Kais. Gesundh.* **15**, 221–228.
- Westley, R. E. (1975). Past, present, and future trends in cultural techniques and oyster production in the State of Washington. *Proc. Annu. Meet.—World Maric. Soc.* **5**, 213–219.
- Whitelegge, T. (1890). Report on the worm affecting the oysters of New South Wales. *Rec. Aust. Mus.* **1**(2), 41–54.
- Wolf, P. H. (1967). Winter mortality hits state's oyster output. *Fisherman, Aust.* **June**, 20–22.
- Wolf, P. H. (1972). Occurrence of a haplosporidan in Sydney rock oysters (*Crassostrea commercialis*) from Moreton Bay, Queensland, Australia. *J. Invertebr. Pathol.* **19**, 416–417.
- Wood, J. L., and Andrews, J. D. (1962). *Haplosporidium costale* (Sporozoa) associated with a disease of Virginia oysters. *Science* **136**, 710–711.

Part VII

Shellfish Diseases and Humans

Among the numerous objectives of this volume—as stated so optimistically in Chapter 1—are these:

- To look at the evidence for effects of environmental degradation on shellfish diseases
- To summarize the information on the economic effects of shellfish diseases
- To review the associations of shellfish diseases and public health

These are all matters of concern to seafood consumers, and each is clearly of stature worthy of a chapter in this concluding section of the volume.

Chapter 14—“Pollution-Associated Diseases”—is an effort to bring together the scattered evidence which suggests some association of pollution and certain diseases and abnormalities of shellfish. Among the diseases with statistically determined relationships with degraded habitats are shell disease and black gill disease of crustaceans; studies on both sides of the Atlantic Ocean have provided data for the relationships. Two latent viral infections (one in oysters and one in shrimp) have been provoked into patency by exposure to forms of pollutant stress, and characteristics of stress responses have been recognized and described in Mollusca and Crustacea. Recent experimental work, using long-term chronic exposure of shellfish to pollutants, has demonstrated pathological effects of toxic chemicals on many species; some examples are the effects of polycyclic aromatic hydrocarbons on mussels, tributyl tin on oysters, and heavy metals on oyster embryos.

Chapter 15—“Economic Effects of Shellfish Diseases”—deals with losses due to disease from the points of view of the producer and the consumer. Supply-side losses result from mortalities, reduced growth, and diminished fecundity. The effects on consumption of seafood products are varied, but center around rejection of abnormal shellfish, re-

sistance to scarcity-induced high prices, and concerns about sanitation and public health. The effects of disease on the profitability of shellfish aquaculture are quantifiable, and several studies of epizootics have led to estimates of major losses to the industries involved. Effects of similar outbreaks in natural populations are more difficult to quantify, but estimates of losses have been attempted (although not often enough—and not by economists).

Chapter 16—“Shellfish Diseases of Public Health Significance”—touches on the sensitive but important matter of the relationships of human diseases to shellfish diseases, and then on the unavoidable broader topic of the relationships of human diseases to shellfish consumption. Remarkably few pathogens of marine molluscs and crustaceans are transmitted directly to people; only *Vibrio parahaemolyticus* and a few trematodes fit this category strictly, although some larval nematodes have been suspected. However, a much larger category—pathogens of humans transmitted *passively* by shellfish—includes agents of cholera, hepatitis, typhoid, and several forms of gastroenteritis, as well as paralytic shellfish poisoning and related toxic effects. Although not technically diseases of shellfish, they may be *transmitted* by shellfish, so they have been included.

The matters considered in this section—pollution, economics, and public health—are all important from a human perspective. Production, marketing, and consumption of seafood are significant activities; the effects of disease on these activities should be clearly understood.

14

Pollution-Associated Diseases

I. INTRODUCTION

The relationship of disease in marine animals and environmental stress is becoming increasingly well established. Human activities—particularly those that result in chemical additions to the coastal/estuarine environment—have increased the potential stresses on shellfish inhabiting those areas. Circumstantial evidence for associations of pollutants with certain shellfish diseases and abnormalities is accumulating.

This chapter attempts to review and evaluate existing information about associations of diseases and marine environmental degradation. Emphasis has been placed on: diseases caused by contaminant stress and facultative pathogens; stress-provoked latent infections; environmentally induced abnormalities, including genetic abnormalities associated with mutagenic and other properties of contaminants; experimentally induced lesions; and contaminant effects on disease resistance.

There are several diseases, particularly shell disease in crustaceans, for which a relationship with pollution seems evident, and there are a number of other diseases or abnormalities for which a relationship with pollution is indicated. Furthermore, there is some evidence that certain latent viral infections may be provoked into patency by environmental stressors.

Disease is a constant concomitant of life for any species, normally removing individuals from the population continuously. Marine animals, are, of course, subject to a wide spectrum of diseases of infectious or noninfectious etiology (disease can be defined in the broad sense as “any departure from normal structure or function of an animal” or as “the end result of interaction between a noxious stimulus and a biological system”).

Dysfunction and death due to the activity of infectious agents constitute the narrower, but often predominant, concept of disease. Infec-

tious diseases—caused by viruses, bacteria, fungi, protozoans, and other pathogenic organisms—are usually prime suspects in the search for causes of mortalities, often to the exclusion of other possible causes. Noninfectious diseases include such phenomena as genetic abnormalities, physiological malfunctions caused by chemical environmental factors, metabolic disorders resulting from nutritional deficiencies, many forms of neoplasia, and a host of others (Sparks, 1972). In many instances it is probably the combination of an infectious agent and an environmental stressor that eventually causes mortality.

Environmental stressors have been implicated in a number of shellfish diseases. Human activity has resulted in the degradation of estuarine/coastal waters near urban centers. We have, for instance, added pesticides and other synthetic chemicals which can, even in low concentrations, drastically affect the physiology of shellfish, and with which the species may have had no previous evolutionary experience. We have added heavy organic loads, in the form of sewage sludge and effluents, which can produce anaerobic or low-oxygen environments and are often accompanied by other contaminants, such as heavy metals, that can interfere with enzymes of the animals and survival of the food organisms they consume. During the past several decades a number of diseases and abnormalities of shellfish have been described that seem to be associated with high pollutant levels. These can be categorized and discussed as:

- Diseases caused by contaminant stress and facultative pathogens
- Stress-provoked latent infections
- Genetic and environmentally induced abnormalities
- Experimentally induced lesions
- Contaminant effects on resistance

This chapter attempts to summarize the present state of knowledge about possible associations of shellfish diseases (infectious or noninfectious) with estuarine and coastal pollution. It should be noted that, to make any firm association of a disease with environmental pollution, there are several basic requirements: (1) knowledge of the history of occurrence of the disease in a particular species in the geographic area of concern; (2) knowledge of the history of occurrence and levels of particular pollutants in that area; (3) a review of the biology, life history, and occurrence of the disease agent in other areas, in other species, and under different environmental conditions; (4) an intensive baseline survey of the current disease and pollution situation, with attention to statistical reliability of the sampling; (5) laboratory and field experimentation with the principal objective of reproducing the disease by exposure to known levels of contaminants; (6) resurveys of the disease and

pollution levels over several years, looking for changes or trends. These requirements have been fully satisfied for few, if any, of the diseases or abnormalities discussed in this chapter.

II. DISEASES CAUSED BY CONTAMINANT STRESS AND FACULTATIVE PATHOGENS

Through a variety of studies, elements of a stress response in molluscs and in crustaceans are emerging. Some are general; some relate specifically to molluscs, some to crustaceans. Responses of molluscs include (Fig. 54):

- Slow growth and reduced “scope for growth” (a measure of the potential for somatic growth and gamete production)
- Decline in condition
- Depression of gametogenic activity
- Destabilization of cellular lysosomal membranes, resulting in autolysis
- High taurine/glycine ratios in gill and mantle tissues

INDICATORS OF STRESS IN BIVALVE MOLLUSCS

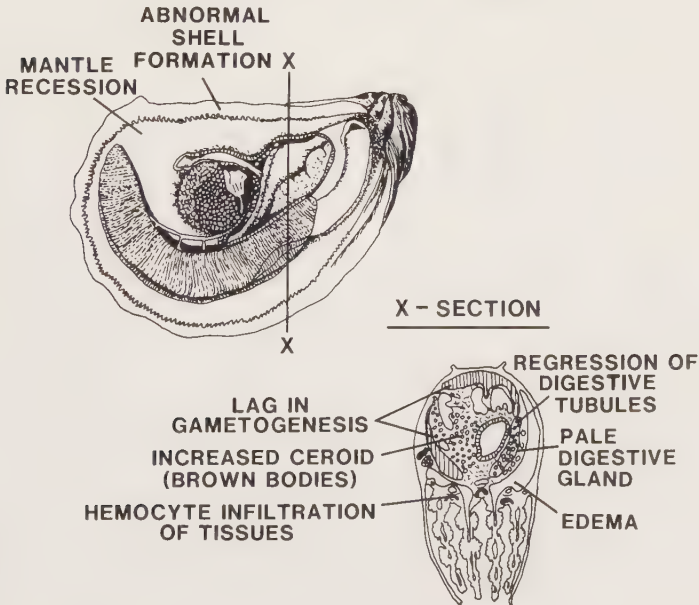


Figure 54. Some responses to stress in bivalve molluscs.

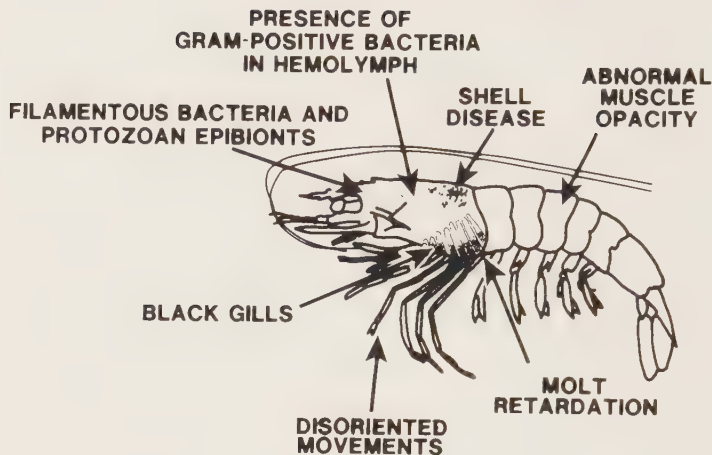


Figure 55. Some responses to stress in crustaceans.

- Regression and atrophy of the digestive tubule epithelium
- Increase in ceroid aggregations
- Hemocyte infiltration of the tissues
- Mantle recession
- Abnormal shell formation

Stress responses of crustaceans include (Fig. 55):

- Black gill syndrome
- Frequent occurrence of shell disease caused by chitinoclastic organisms
- Abnormal muscle opacity
- Abnormal growth of epibionts—especially filamentous bacteria and ciliate protozoans
- Molt retardation
- Presence of gram-positive bacteria in the hemolymph
- Abnormal, disoriented behavior
- Clotting of hemolymph (as a response to the presence of gram-negative endotoxin)

Although most studies of the physiological and morphological consequences of stress have emphasized vertebrates, there are indications that counterpart phenomena may exist in invertebrates as well. For Mollusca several publications have provided evidence for a generalized stress response syndrome. Jeffries (1972), working with clams, *Mercentaria mercenaria*, found a shortened life span, mass mortalities, a reduc-

tion in total amino acid and carbohydrate content, and shell invasion by *Polydora* in stressed clams. He considered amino acid ratios, particularly the taurine/glycine ratio in gill and mantle tissues, to be the best indicators of stress. Ratios above 3 indicated stress, with ratios above 5 an indication of acute stress. High taurine ratios were also observed by Feng *et al.* (1970) in oysters infected with the trematode *Bucephalus*. In Jeffries' study, exposure of clams to petroleum resulted in black tar-like concretions in amebocytes and in kidney tissues. The concretions collected in the renal sac and plugged the kidney tubules.

Other indices of stress in molluscs have been proposed—particularly a reduced scope for growth (Bayne, 1975; Bayne *et al.*, 1979) and a reduced ratio of oxygen consumed to nitrogen excreted (Corner and Cowey, 1968; Bayne and Scullard, 1977). Working with mussels, Bayne and associates observed a decline in condition and a depression of gametogenic activity under stress conditions. Other studies, by Moore (1976, 1977), related the degree of cell damage due to destabilization of lysosomal membranes to degrees of stress.

Often, the outcome of pollution stress is infectious disease, usually with a facultative microbial agent involved. Sometimes, though, the infectious process is not clearly defined, so that the same etiological agent may not be isolated consistently, or no infectious agent may be associated with the abnormality in every case. Some examples follow.

A. Growth Retardation in Molluscs

Reduction in growth rates and an increase in mortality rates of molluscs are common phenomena associated with polluted habitats (Fig. 56). Several studies have demonstrated these effects. The growth rates of soft clams, *Mya arenaria*, declined by 65% when clams were transplanted to an oil-polluted site on the Maine coast (Dow, 1975). Survival at the polluted site was only 12%, compared with 78% at a reference site.

In another study slow growth and high mortality in black abalone, *Haliotis cracherodii*, from the California coast was reported by Young (1964). Comparing populations at the Los Angeles County sewer outfall site with those from Santa Catalina Island, the polluted outfall site yielded samples of abalone which were starving, had eroded shells, and died at a much higher rate than did their Island relatives. Individuals transplanted from the clean site to the outfall area failed to grow and eventually died from undetermined causes. Findings were consistent with the results of examining fish of several species from outfall sites in California waters, in which lesions, emaciation, oral tumors, and exophthalmia were noted (Young, 1964).

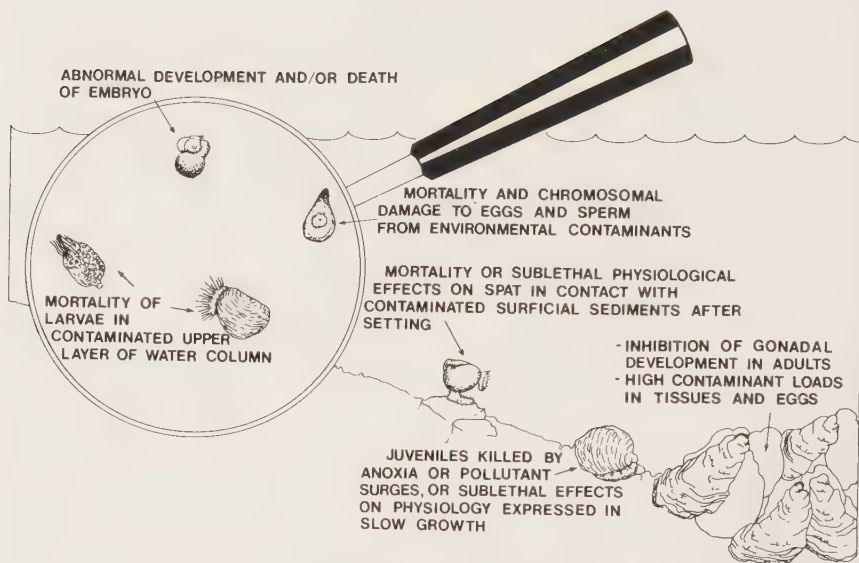


Figure 56. Contaminant effects on oyster life cycle stages.

B. Effects of a Large-Scale Oil Spill on Molluscs

The tanker *Amoco Cadiz* was wrecked on the Brittany coast of France in 1978, spilling about 233,000 tons of crude oil. Effects on the oyster populations of the area have been examined since that catastrophe, through a combination of field surveys and experimental introductions of clean oysters (*Ostrea edulis* and *Crassostrea gigas*) (Berthou *et al.*, 1987). Some of the findings that relate to pollution and disease are these:

1. Mortalities of 20–50% occurred in the most heavily polluted sites in the first 3 months after the spill.
2. Lesions, principally necrosis or atrophy of the digestive tract epithelium, were observed frequently during 1978 and in several succeeding years, gradually falling to background levels by 1983.
3. No evidence was found for a relationship of oil pollution with prevalences or effects of two protozoan pathogens—*Marteilia refringens* and *Bonamia ostreae*—in *O. edulis*.
4. Severe gonadal lesions were seen in 1978 in the form of destruction of the duct epithelium, intense inflammatory reaction, and final atrophy. Total suppression of the reproduction of *O. edulis* was postulated for 1978 (Neff and Haensly, 1982).

5. Hemocytic neoplasms have been reported in *O. edulis*, but prevalences did not increase following the contamination event. Neoplasms with prevalences up to 40% were observed in cockles, *Cerastoderma edule*, in 1983, but a relationship with pollution was not established (Poder and Auffret, 1986).
6. Residual hydrocarbon levels in oysters in 1985 were still two to five times the values found in an unpolluted reference site, but histopathological signs, seen earlier in the form of digestive gland and gonadal lesions, had disappeared (Berthou *et al.*, 1987). "Indifference" of oysters to chronic petroleum pollution, as measured by the results of histopathological examination, was suggested by the authors, but this is not at all consistent with findings from experimental exposures of another species of oyster (*Crassostrea virginica*) to chronic low levels of crude oil, in which severe alterations in the histology of the digestive tract and the gonads were seen (Barszcz *et al.*, 1978).

The continuing examination of the effects of this single massive oil pollution event on molluscan disease has thus provided useful information on short-term as well as long-term damage.

C. Shell Disease of Crustacea

Associated with badly degraded estuarine and coastal waters is a disease condition in Crustacea commonly referred to as shell disease, exoskeletal disease, or shell erosion. This can be considered in some ways at the invertebrate analog of fin erosion in fish.

Lobsters (*Homarus americanus*) and rock crabs (*Cancer irroratus*) from grossly polluted areas of the New York Bight were found by Young and Pearce (1975) to be abnormal, with appendage and gill erosion a most common sign. Skeletal erosion occurred principally on the tips of the walking legs, the ventral sides of chelipeds, exoskeletal spines, gill lamellae, and around areas of exoskeletal articulation, where contaminated sediments could accumulate. Gills of crabs and lobsters sampled at the dump sites were usually clogged with detritus, possessed a dark brown coating, contained localized thickenings, and displayed areas of erosion and necrosis (Fig. 57). Similar disease signs were produced experimentally in animals held for 6 weeks in aquaria containing sediments from sewage sludge or dredge spoil disposal sites. Initial discrete areas of erosion became confluent, covering large areas of the exoskeleton, and often parts of appendages were lost. The chitinous covering of the gill filaments was also eroded, and often the underlying tissues became necrotic.

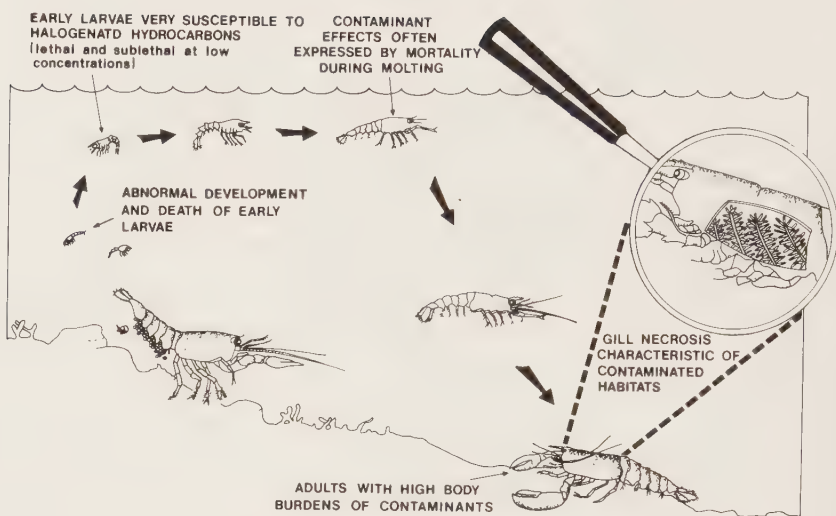


Figure 57. Contaminant effects on crustacean life cycle stages.

In a related study Gopalan and Young (1975) examined shell disease in the caridean shrimp, *Crangon septemspinosa*, an estuarine and coastal food chain organism common on the east coast of North America and important in the diets of bluefish, weakfish, flounders, sea bass, and other economic species. Examinations of samples of *Crangon* from the New York Bight disclosed high prevalences (up to 15%) of eroded appendages and blackened erosions of the exoskeleton. The disease condition was only rarely observed at other collecting sites (i.e., Beaufort, North Carolina, and Woods Hole, Massachusetts). Histological examination of diseased specimens produced findings similar to those of Young and Pearce (1975) with crabs and lobsters. All layers of the exoskeleton were eroded, affected portions were brittle and easily fragmented, cracking and pitting of calcified layers occurred, and underlying tissues were often necrotic. Laboratory experiments using seawater from the highly polluted inner New York Bight resulted in appearance of the disease in 50% of individuals. Erosion was progressive, crippled individuals were cannibalized, and eroded segments of appendages did not regenerate after ecdysis. No disease signs developed in control animals held in artificial seawater.

A German study of the effects of industrial wastes on the brown shrimp, *Crangon crangon* (Schlotfeldt, 1972), disclosed a high prevalence of so-called black spot disease, with signs very similar to those seen in *C. septemspinosa* from the New York Bight. Juvenile and adult shrimp from

the Fohr Estuary had black areas of erosion on the carapace and the appendages, with necrosis of the underlying tissues, and, frequently, missing terminal segments of appendages. The disease condition varied in prevalence seasonally, with a peak of 8.9% in the summer. Lesions persisted and worsened after ecdysis, and experimental exposure to detergent accelerated the course of the disease.

A recent study of shell disease and black gill disease in lobsters from Massachusetts waters showed similar trends for both diseases, with highest prevalences in samples from the most polluted sites—particularly Boston Harbor and Buzzards Bay (Estrella, 1984). Mortalities were not observed, but population impacts were considered likely.

Black gill disease was also examined in rock crabs, *Cancer irroratus*, from the Atlantic coast of North America (Sawyer, 1982; Sawyer *et al.*, 1983, 1984). High prevalences were associated with sewage and/or dredge spoil dumping areas where environmental degradation was worst. Prevalences of black gill disease were as high as 30% in some samples from the New York Bight apex. The etiology of the disease is clearly complex, but it involves the presence of black silt between lamellae and the presence of epiphytic and epizootic organisms. Foci of black discoloration were found to be areas in which several adjacent lamellae were dead and necrotic, with accompanying melanization and necrosis of the gill tissue (Sawyer *et al.*, 1983).

Shell disease of Crustacea has been observed in many species and under many conditions, both natural and artificial (Rosen, 1970; Sidermann, 1979). Shell erosion seems to involve activity of chitinoclastic microorganisms, with subsequent secondary infection of the underlying tissue by facultative pathogens. Initial preparation of the exoskeletal substrate by mechanical, chemical, or microbial action probably is significant; thus, high bacterial populations and the presence of contaminant chemicals in polluted environments, as well as extensive detrital and epibiotic fouling of the gills, could combine to make shell disease a common phenomenon and a significant mortality factor in crustaceans inhabiting degraded environments.

There is much room for study in this cloudy territory at the boundary between infectious and noninfectious disease processes, as exemplified by shell erosion. This is the area in which environmental stress and facultative microorganisms exert their impacts; high bacterial populations in eutrophic waters interact with exposed, injured, or chemically modified surface membranes; epibiotic fouling organisms can assume pathogenic roles; and nonspecific lesions, such as exoskeletal erosions, can occur in epizootic proportions.

III. STRESS-PROVOKED LATENT INFECTIONS

Recently published accounts of two viral diseases of marine invertebrates indicate that latent infections may be provoked into patency by environmental stress. One, a *Baculovirus* infection of pink shrimp, *Penaeus duorarum*, was first recognized in stressed laboratory populations (Couch, 1974b, 1976). The other, a herpes-like viral infection of oysters, was discovered in a population held in a heated power plant effluent in Maine (Farley *et al.*, 1972).

An association of shrimp viral disease and low-level chronic exposure to pollutant chemicals has been explored at the Gulf Breeze (Florida) Environmental Research Laboratory of the U.S. Environmental Protection Agency (Couch, 1974a, 1978). In this work a viral disease of pink shrimp caused by *Baculovirus penaei* reached patent levels and caused mortalities of 50–80% in shrimp exposed to the polychlorinated biphenyl (PCB) Aroclor 1254 and the organochlorine insecticide Mirex (Nimmo *et al.*, 1971; Couch and Nimmo, 1974a,b; Couch, 1974a,b, 1976). Other experiments in which the shrimp were crowded, but not exposed to chemicals, resulted in similar enhancement of viral infections, indicating that environmental stress may be an important determinant of patent infections. The viral infections have been found subsequently in brown and white shrimp (Overstreet and Howse, 1977; Couch, 1978).

Couch and Courtney (1977) proposed an elaborate and unique conceptual scheme to utilize the shrimp virus for interactive bioassays for chronic sublethal effects of contaminants. The authors pointed out that there are a number of possible interactions of host, pathogen, and chemical stressors: change in the resistance of shrimp to the virus, the enhancement of widespread latent infections in the shrimp population, change in the virulence of the virus, and losses of diseased shrimp by cannibalism. Criteria developed by Couch and Courtney for interaction included increased viral prevalence in stressed populations (as indicated by the numbers of viral inclusion bodies in infected animals), increased infection intensity in stressed individuals, increased mortality in stressed populations, and greater cytopathic effects in infected and stressed individuals. The shrimp viral infection has great potential for elucidating the effects of pollutants on host–pathogen relationships.

An association of high environmental temperatures with high disease prevalence (or disease enhancement) in molluscan shellfish sampled from thermal effluents has been made recently. Farley *et al.* (1972) described a lethal herpes-type viral disease of oysters held in heated discharge water in Maine. The disease, which apparently existed at a low enzootic level in oysters growing at normal, low environmental tem-

peratures (12–18°C summer temperatures), proliferated in oysters maintained at elevated temperatures (28–30°C) and produced mortalities in those populations. Intranuclear inclusion bodies, containing viral particles, characterized advanced infections. Mortalities of oysters held at higher temperatures were correlated with a greater prevalence of the viral inclusions. Elevated water temperatures were considered by the authors to favor spread of the infection or to activate latent infections, or both.

IV. GENETIC AND ENVIRONMENTALLY INDUCED ABNORMALITIES

A. Neoplasia and Related Disorders

Although neoplasia has been studied most extensively in humans and in laboratory mammals, the existence of tumors in shellfish has been recognized for a century (the first oyster tumor, for example, was reported by Ryder in 1887). The possible role of environmental chemical factors in induction of neoplasms in shellfish is uncertain, but there is some limited information.

Yevich and associates (Barry and Yevich, 1972, 1975; Yevich and Barszcz, 1976, 1977) examined the occurrence of neoplastic growths in the soft-shell clam, *Mya arenaria*, in relation to petroleum contamination. Gonadal and hematopoietic neoplasms were observed in animals collected from two chronically contaminated sites on the Maine coast, with prevalences up to 29% in certain samples. Yevich and Barszcz (1976) stated that “no tumors similar to those described [from the petroleum contaminated area] have been encountered in animals collected from any other area.” They described the scope of their study as “several thousand animals from all coastal areas of the United States.”

Then, beginning in 1976 a greatly expanded study of the prevalence of neoplasms¹⁰ in *M. arenaria* was instituted, to determine whether they

¹⁰The terms used for the proliferative disorders of bivalve molluscs can be confusing, principally because there is uncertainty about whether they are really neoplastic, and also because the origin of the abnormalities is uncertain. Thus, “hemocytic neoplasm” has been applied by a number of workers, while the more conservative “sarcomatoid proliferative disease” is preferred by others, especially in view of recent information from monoclonal antibody studies indicating a lack of antigenic identity of the abnormal cells and hemocytes. Part of the problem is a lack of agreement on criteria for neoplasia in invertebrates. Pauley (1969) suggested that “infiltration, invasion, or replacement of normal tissues by actively mitotic atypical cells indicates a potentially neoplastic condition.” Dawe (1969) provided a tabulation of 36 characteristics that could be used to identify and classify neoplasms.

were associated with varying types and degrees of environmental hydrocarbon pollution (Brown *et al.*, 1976, 1977; Walker *et al.*, 1981). Ten coastal sites from Maine to Rhode Island were selected, based on the existence of various kinds and intensities of pollution. Examination of 1829 clams disclosed neoplasia in 159 (8%), but in only five of the ten sites. Prevalences varied remarkably among sites: from 0 to 64%. Gonadal and haematopoietic neoplasias were found; the haematopoietic neoplasms were observed to cause poor condition and mortality. An interesting geographic variation was seen, with only haematopoietic neoplasms found in southern New England and predominantly gonadal neoplasms found in northern New England. The relationship of clam neoplasms to oil pollution was not clarified by the study. In the words of the authors (Brown *et al.*, 1977), "There is a surprising dichotomy of results from petroleum-derived hydrocarbon (PDH)-polluted sites. Clams from some polluted sites had no neoplasms, whereas other PDH-polluted sites had a high prevalence of neoplasms. These results suggested that the type and degree of hydrocarbon pollution are possibly related to the frequency of neoplasms and other lesions in *Mya*, but they are by no means the only causative factors." The authors went on to state that "the possibility that neoplastic disease in *Mya* has a genetic base or is a result of an infectious agent cannot be excluded by our results" (emphasis added).

Then, in 1979 and in 1980 the experimental induction of haematopoietic neoplasms in clams was reported (Brown *et al.*, 1979; Brown, 1980; Appeldoorn and Oprandy, 1980). Exposure of normal clams to water passed over populations of neoplastic clams, with or without the presence of petroleum hydrocarbons, resulted in significant levels of neoplasia in experimental animals. Prevalence exceeded 70% in one instance. This was the first reported transmission of neoplasia in marine invertebrates and strongly indicated the presence of an infectious agent. Viruslike particles were found in neoplastic clams, and they were reported to produce neoplasia when injected into normal clams. The re-isolation of virus was also reported. Results of the experiment also indicated that stress enhanced susceptibility of clams to the disease.

Another step in the clam neoplasia story was published by Oprandy *et al.* (1981). A viral etiological agent was described and was reported to be similar to B-type retroviruses. Purified virus, when injected into clams, was reported to cause tumors within 2 months. No direct correlation between disease prevalence and the type or extent of environmental pollution was demonstrated in any of the experiments, but the role of petroleum hydrocarbons as stressors was not discounted.

There are, of course, many other reports of neoplasms in molluscs [reviewed by Pauley (1969), Dawe and Harshbarger (1969), and Farley

(1976)]. Haematopoietic neoplasms have been observed in a number of species in addition to *Mya arenaria* (Mix *et al.*, 1979; Farley, 1969b; Farley and Sparks, 1970; Frierman, 1976). Yevich and associates (Yevich and Barry, 1969; Barry and Yevich, 1972) have also described gonadal neoplasms in quahogs, *Mercenaria mercenaria*, from Narragansett Bay, Rhode Island. Samples collected in 1968, 1969, and 1970 had tumor frequencies of 0.2, 2.3, and 2.7%, respectively. In another study (Christensen *et al.*, 1974) epizootic neoplasms (up to 10% prevalence) were found in a localized population of the clam *Macoma balthica* from a tributary of Chesapeake Bay. The neoplasms were invasive and systemic, with initial foci in the gill epithelia. Holding experiments indicated that the disease was usually fatal. No association with environmental factors was made.

Epizootic neoplasms with a possible environmental etiology were reported in several molluscan species from Yaquina Bay, Oregon (Farley, 1969a; Farley and Sparks, 1970; Mix, 1975, 1976; Mix *et al.*, 1977). Blue mussels (*Mytilus edulis*), native oysters (*Ostrea lurida*), and two species of *Macoma* were affected, and winter mortalities were associated with the disease. In a related chemical study (Mix *et al.*, 1979) mussels from polluted Oregon estuaries were found to have significant body benzo[a]pyrene burdens and neoplasms, while those with low or undetectable levels of benzo[a]pyrene had no neoplasms. Other than these reports, there are many others that fail to disclose any association or relationship of molluscan neoplasia and pollution.

Mussels (*M. edulis*) from British waters were also found to have a "proliferative atypical haemocytic condition" (Lowe and Moore, 1978). Described as "infiltration and replacement of the vesicular connective tissue by enlarged, atypical, mitotically active, basophilic, haemocyte-like cells," the disorder was seen in varying degrees of severity. Advanced cases were characterized by extensive replacement of connective tissue and degeneration of digestive gland cells. The condition is similar to that seen in mussels from Oregon, and samples from a hydrocarbon-polluted site in southern England (Plymouth) disclosed a prevalence of 1.6%. Sediments from the collection site contained several aromatic hydrocarbons, but the authors concluded that "any speculation about the etiology of the [proliferative] condition described must remain inconclusive."

A nonneoplastic inflammatory lesion, possibly a response to pollutants, has also been reported in mussels, *M. edulis*, from England (Lowe and Moore, 1979). Described as granulocytomas, the lesions consisted primarily of degenerating granulocytes, which were encapsulated and caused vascular occlusions in the digestive gland and mantle. No evidence of mitotic figures or parasite invasion was seen, and the lesions were progressive, causing atrophy and autolysis of surrounding

tissues—suggesting a possible fatal outcome. The granulocytomas were common (up to 26% prevalence) in mussels from polluted sites, but were not found in samples from a clean reference site.

The only reported tumorlike growth in crustaceans that may have a relationship with pollution is a hamartoma (a nonneoplastic overgrowth of muscle tissue) seen in postlarval brown shrimp (*Penaeus aztecus*) and white shrimp (*Penaeus setiferus*) from a polluted Mississippi estuary (Overstreet and VanDevender, 1978). The growths penetrated the ventral abdominal integument and seemed similar to a tumorlike growth reported earlier in brown shrimp (Sparks and Lightner, 1973). The hamartomas were not seen in extensive samples of postlarvae from non-polluted sites, nor were they seen in juvenile or adult shrimp.

B. Shell Abnormalities in Molluscs

Pacific oysters, *Crassostrea gigas*, were introduced to the coast of France in the late 1960s and to the east coast of England in the early 1970s. By the mid-1970s a serious problem of reduced growth and malformed shells was apparent, and the cause was found to be organotin compounds used on boats in antifouling paints. Abnormally thickened shells in exposed populations of oysters had an open laminar structure, a formation described as "chambering," sometimes with a proteinaceous gel secreted between the layers. In one French study such abnormalities in shell calcification reached 90% during 1980–1982 in the Bay of Arcachon, but fell to 40% in 1983–1985, after a ban on the use of tributyl tin in antifouling paint was imposed (Alzieu *et al.*, 1982, 1986). The severity of the deformations and the tissue levels of tin also decreased during this period, indicating the chemical etiology of the condition. The extreme sensitivity of oysters to tributyl tin is apparent from findings that concentrations of only 50 ng/liter could induce shell malformations (Alzieu *et al.*, 1986). In the British study, *C. gigas* spat cultured in the presence of low, environmentally feasible levels of the contaminant, grew more poorly than did controls and developed pronounced thickening and chambering of the upper shell valve (Waldock and Thain, 1983). Higher levels of exposure resulted in increasing percentages of mortalities. [It seems relevant to note here that experimental exposure of crabs, *Uca pugilator*, to tributyl tin also produced morphological abnormalities in the form of limb deformities (Weis *et al.*, 1987).]

C. Genetic Abnormalities

The mutagenic properties of a number of chemical contaminants, including heavy metals, pesticides, and petroleum-derived polycyclic hydrocarbons, have been demonstrated in experimental studies with

terrestrial animals (Huberman, 1975; Longwell, 1975). It is likely that marine organisms will respond to mutagens in species-specific ways and with differing sensitivities.

An indirect test for the presence of mutagens in the marine environment was reported by Parry *et al* (1976). *Mytilus edulis* were sampled from polluted and unpolluted waters of the United Kingdom, and extracts of their tissues were tested for the ability to induce genetic changes in bacterial and yeast cultures. Significant increases in mutation rates for specific gene loci characterized cultures exposed to extracts of mussels from polluted waters, but not those from clean waters—providing evidence for the presence of mutagens that had been concentrated in the tissues of mussels from polluted areas. The chemical nature of the mutagens was not identified, except that the mussels came from areas with heavy industrial pollution.

Other microbial assay systems for the detection of environmental mutagens and carcinogens have been developed (see the summary by Evans, 1978). Many are based on the bacterial test for mutagens developed originally by Ames (1972); many could have relevance to the assessment of pollutants in marine animals. One of the most promising assay approaches is the sister chromatid exchange technique, which has been used to measure mutagenicity in mussels (*M. edulis*) exposed to environmental mutagens (Dixon and Clarke, 1982). The degree of chromosomal damage is measured by differential staining properties and is an indicator of mutagen concentration (Latt *et al.*, 1981; Dixon, 1983).

Looking beyond the process to the results of exposing developing embryos to pollutants, several studies have made use of commercial species, including oysters (*C. virginica*) and periwinkles (*Littorina saxatilis*). Heavy metal toxicity to oyster embryos was examined by Calabrese *et al.* (1973), principally to determine LC⁵⁰ concentrations. A later study of developmental abnormalities in embryos of periwinkles disclosed a direct relationship with the degree of pollution stress to which the adult population had been exposed (Dixon and Pollard, 1985). Collections from polluted sites on the coast of South Wales produced much higher prevalences of deformed embryos than did those from a clean site. A possible infectious agent was postulated, but not demonstrated, leaving unresolved the question of possible complex etiology for the disorders seen in embryonic development.

V. EXPERIMENTALLY INDUCED LESIONS

Early experimental exposures of estuarine and marine animals to contaminants usually had the purpose of determining lethal dosages from

acute exposures. More recently, attention has been redirected to chronic sublethal toxic effects, expressed in behavioral, physiological, or cytological responses to specific contaminants. A wealth of experimental data and interpretations has accumulated; only a small sampling is included here.

Experimental cadmium exposures can cause gill lesions in shrimp, as reported in recent papers by Nimmo *et al.* (1977) and by Couch (1977). Exposure of pink shrimp to 763 $\mu\text{g/liter}$ of cadmium for 15 days resulted in a black gill condition characterized by necrosis of all cell types in the distal gill filaments, with coincident appearance of black granules in the cytoplasm and some hemocyte infiltration at the bases of the necrotic filaments. Couch suggested that the black deposits could be a metallic sulfide or even cadmium. He further pointed out that the distal filament tissue has been postulated to have detoxifying, as well as osmoregulatory and respiratory, functions, so that cell death could result from cadmium filtration and accumulation as part of a detoxification process.

An interesting study of pathology in American lobsters, *H. americanus*, was made following disclosure of severe yellow phosphorus industrial contamination of Placentia Bay, Newfoundland (Aiken and Byard, 1972). Experimental lobsters, exposed to phosphorus-contaminated sediments in aquaria, exhibited degenerative changes in antennal glands and in all cell types in the hepatopancreas, as well as massive coagulation of hemolymph.

Histopathological effects of petroleum on bivalve molluscs are varied in the extreme. Effects, particularly on the gill epithelium, have been observed by Barry *et al.* (1971), Jeffries (1972), LaRoche (1972), Clark *et al.* (1974), and Gardner *et al.* (1975). Fries and Tripp (1976) and Tripp *et al.* (1984) found damage to the gill epithelium in hard shell clams, *Mercenaria mercenaria*, exposed to as little as 1 ppm phenol. Vaughn (1973), however, found little histopathology after chronic exposures of oysters to No. 2 fuel oil. Stainken (1975) found that exposure of soft-shell clams to No. 2 fuel oil at winter seawater temperatures (4°C) for 28 days had little histopathological effect, beyond signs of starvation (glycogen depletion and vacuolization of the digestive diverticula cells), and a generalized hemocytosis, even at 100 ppm. No mortalities occurred, and exposure concentrations dropped rapidly, possibly because much of the oil was trapped in mucus as part of the mucociliary feeding mechanism and ejected from the clam.

A recent study of the morphological, biochemical, and physiological effects of chronic exposure to low-level ("environmentally relevant") petroleum contamination on lobsters disclosed that increased gill pigmentation was the most striking response (Payne *et al.*, 1983). "Gill browning," as the condition was called, was proposed as a general indi-

cator of stress in crustaceans, since it is similar to responses to cadmium exposure (Nimmo *et al.*, 1977) and those to fungal and protozoan infections (Couch, 1978). Histological examination of lobster gill tissues disclosed various stages of occlusion of filament hemolymphatic channels, which would lead to impaired respiratory and osmoregulatory functions. Use of gill browning was suggested as a monitor for point sources of petroleum contamination of estuarine/coastal waters (Payne *et al.*, 1983).

Exposure of oysters (*Crassostrea virginica*) to the carcinogens benzo[a]pyrene and 3-methylcholanthrene resulted in inflammation of the connective tissue surrounding blood vessels and proliferation of a cell type closely resembling those found in sarcomatoid neoplasms of natural populations of oysters and other bivalves (Couch *et al.*, 1979). The abnormal cells exhibited mitotic activity and were larger than normal oyster hemocytes—which are predominant characteristics of neoplastic cells. The authors did not, however, consider the lesions to be unequivocal neoplasms, and the experimentally induced lesions were not as extensive as those seen in wild populations of bivalves.

Pesticides and related compounds can produce tissue pathology in invertebrates. Oysters exposed chronically to 3 ppb dichlorodiphenyl-trichloroethane (DDT), toxaphene, and parathion exhibited variable lesions, including hemocytic infiltration or hyperplasia of the gonadal germinal epithelium, necrosis of the digestive tubule epithelium, and edema (Lowe, *et al.*, 1971). In another study chronic exposure of oysters to 5 ppb PCBs produced atrophy of the digestive epithelium, hemocytic infiltration, and degeneration of the vesicular connective tissue (Lowe, *et al.*, 1972).

A number of experimental studies have demonstrated pathological effects of toxic organic chemicals on the tissues and organs of crustaceans. Gill edema and progressive necrosis of filaments in the crustacean *Gammarus oceanicus* resulted from exposure to sublethal concentrations of PCBs (Wildish, 1970). Examination of pink shrimp, exposed experimentally to PCBs, disclosed a variety of nonspecific tissue changes, especially in the hepatopancreas (Couch *et al.*, 1974). Histological changes included lysis of the hepatopancreatic epithelium, nuclear pyknosis, vacuolization of secretory cells, and a variety of ultrastructural changes in absorptive cells.

Exposure of grass shrimp, *Palaemonetes pugio*, to dithiocarbamate biocides produced pronounced cellular changes in the antennal glands, hepatopancreas, and midgut (Doughtie and Rao, 1983a,b). Associated with these changes were progressive blackening and degeneration of gill lamellae—the black gill syndrome (Fig. 58).

It might be a good idea at this point to emphasize that the black gill



Figure 58. Black gills in juvenile shrimp. Shown are the gross appearance (top) and a wet mount of a gill process (bottom). (Courtesy of Dr. D. V. Lightner.)

syndrome seems to be an important hemocyte-mediated defensive response of Crustacea to chemical or physical trauma or to pathogen invasion. It results in the sequestering and subsequent removal during ecdysis of toxic substances or microbial invaders. It also results in truncated gill filaments, with a consequent reduction in gas and ion exchanges.

The essence of the syndrome is the formation of apical cellular plugs consisting of hemocytes, formed in response to gill trauma or systemic microbial infection. Phagocytized material is aggregated in gill apices. Gill blackening has been reported as a consequence of protozoan and fungal infection (Couch, 1978; Lightner, 1975) and cadmium exposure (Couch, 1977; Nimmo *et al.*, 1977). In one study (Doughtie and Rao, 1983b) several defensive responses to toxin exposure were proposed. Included were:

1. Formation of a hemocyte plug in the apices of gill lamellae
2. Phagocytosis of cellular debris by circulating granular hemocytes
3. Phagocytosis of cellular debris by fixed hepatopancreatic cells
4. Encapsulation by circulating hemocytes of degenerated tissues resulting from the action of toxins
5. Darkening of hemolymph in gill lamellae and in areas where degenerative changes were occurring
6. Attainment and maintenance of "heterostasis"—an abnormal level of equilibrium in the continued presence of a toxic stressor—promoted by an enhanced detoxifying activity of the hepatopancreas and excretory activity of the antennal gland

These responses, or their extrapolations, may be important to the survival of crustaceans in degraded habitats.

The use of histopathology in recognizing stress from environmental contaminants has been proposed, and some evidence for a "stress syndrome" in invertebrates has been presented. Thus, Nascimento *et al.* (1986), studying a mortality of oysters to which contamination may have contributed, observed the following histological changes:

- Nonspecific perivascular hemocytosis in mantle connective tissue
- Increased occurrence of ceroid pigment foci
- Metaplasia of epithelial cells of the digestive diverticula
- Decrease in gametogenesis
- Reduction in stored glycogen
- Edema

In an earlier study, this one with clams (*Mercenaria mercenaria*), Jeffries (1972) found that exposure of clams to petroleum resulted in black

tar-like concretions in hemocytes and in the kidney tissues. The concretions collected in the renal sac and plugged the kidney tubules.

Other histological evidence exists for stress effects on molluscs. Bayne *et al.* (1978, 1979), working with mussels (*Mytilus edulis*), observed a decline in condition and a depression of gametogenic activity under stress conditions, and studies by Moore (1976, 1977) related the degree of cell damage due to destabilization of lysosomal membranes to degrees of stress.

In a later paper Moore (1985) reviewed the cellular responses of marine molluscs to pollutants, emphasizing

1. Pollutant-induced alterations in the epithelial cells of the digestive tubules, often leading to thinning or atrophy (this response has also been seen in *Mytilus edulis* and in *Mercenaria mercenaria* exposed to petroleum and benzo[a]pyrene, respectively)
2. Presence of proliferative neoplastic cells in *M. edulis*, possibly linked to exposure to polycyclic aromatic hydrocarbons
3. Occlusion of hemolymph sinuses by granulocytomas, seen in *M. edulis* from polluted sites
4. Alterations in the boundary membranes of lysosomes of digestive cells, leading to enlarged lysosomes and increased autolytic activity within the cell, seen in *M. edulis* exposed to oil-derived contaminants
5. Extracellular release of lysosomal enzymes
6. Induction of cytochrome P-450 reductase, a component of the microsomal detoxification system, in hemocytes and hepatopancreatic cells after exposure to organic pollutants
7. Enhancement of phagocytosis by oyster hemocytes after exposure to heavy metal ions

Moore pointed out that much remains to be learned about cellular and subcellular reactions to pollutants, but that changes of the type listed can serve as indicators of the presence of toxic chemicals.

In summary, a variety of pathologies may be induced in invertebrates by exposure to contaminants, but despite the interesting findings just discussed, histopathology must still be described as a mediocre indicator of pollution stress, because it lacks the necessary sensitivity and specificity. Sensitivity of histopathology in indicating pollutant-induced injury can be enhanced by focusing on a single cell type that may be susceptible to injury by toxic chemicals, as was pointed out by Moore (1985); but the results may still be subject to high variability because of influences of environmental factors other than pollution—as well as physiological factors that may vary cyclically or irregularly.

VI. CONTAMINANT EFFECTS ON DISEASE RESISTANCE

Among the invertebrates, indirect evidence for reduction of disease resistance caused by contaminant exposure is available and has already been discussed in previous sections on crustacean shell disease and shrimp viral disease. Direct experimental evidence exists as well, but it is scarce. Fries and Tripp (1976) exposed hard clams, *M. mercenaria*, to phenol and found damage to gill and digestive tract epithelia—tissues which are considered to be important components of internal defense mechanisms. The authors suggested, but did not demonstrate, that phenol-treated clams may be more susceptible to microbial infections than normal ones. A mechanism for increased susceptibility of clams to bacterial infection was proposed by Anderson *et al.* (1981) based on chronic (18-week) exposure to benzo[*a*]pyrene, pentachlorophenol, and hexachlorobenzene. Most of the exposed clams showed significantly impaired ability to clear injected bacteria (*Flavobacterium* sp.), implying that resistance to bacterial invasion was decreased by exposure to xenobiotics. The deficiency in bacterial clearance was directly proportional to tissue levels of the chemicals used (some clams with high tissue burdens were unable to clear bacteria). Bacterial clearance may result from activity of cellular and humoral factors, and a number of these have been demonstrated in hard clams. Phagocytic activity was reduced after exposure to pollutants (Fries and Tripp, 1980), as was the presence of bacterial agglutinins and lysins (Arimoto and Tripp, 1977; Anderson, 1981). Pollutant exposure has thus far been demonstrated to affect phagocytosis in hard clams, but effects on humoral factors have not been reported.

Pollutants may affect the cellular defenses of molluscs in a positive way as well. Phagocytic activity of oyster hemocytes has been reported to be enhanced by exposure to heavy metals (Cheng and Sullivan, 1984). In studies of mussels exposed to graded doses of copper ions, a dramatic increase in circulating granulocytes was seen, as was an increase in hemolymph lysozyme, total protein, and proteolytic activity (Pickwell and Steinert, 1984). Binding and sequestering of excess copper was a later phenomenon. In other studies with invertebrates, Telford (1968, 1974) demonstrated that environmental stress increased blood glucose levels in lobsters, *Homarus americanus*, and crayfish, *Cambarus clarkii*.

Generally, though, there is little information available about the effects of toxic chemicals on disease resistance in molluscs and in crustaceans. Reaching out to other phyla, exposure of a polychaete (*Glycera dibranchiata*) to pentachlorophenol and hexachlorobenzene induced release of a bactericidal glycoprotein, but decreased the effectiveness of phagocytes (Anderson *et al.*, 1984). The authors suggested that the re-

lease of the bactericidal glycoprotein from coelomocytes was similar to macrophage activation in vertebrates and that the recognition and binding mechanisms of phagocytes were the components affected by exposure to pollutants (possibly leading to the decreased ability to recognize and destroy pathogens). Humoral resistance factors, known to exist in the worm (Anderson, 1980; Anderson and Chain, 1982), were unaffected by the pollutant dosages used.

There is some experimental evidence for a causal relationship between specific pollutant chemicals and fungal parasitization of shellfish. In one study oysters exposed to pesticides (e.g., DDT, toxaphene, and parathion) became infected with a mycelial fungus (not further identified) that caused lysis of the mantle, gut, gonads, gills, visceral ganglion, and kidney tubules (Lowe, *et al.*, 1971). None of the control oysters became infected, indicating a role for one or several of the pesticides in altering the host-parasite relationship of the oysters and the fungus. Presence of fungal infections made it difficult to differentiate the histopathological effects of pesticide exposure from those due to the parasite.

Other investigators have suggested that chronic pollution may place a stress on the animal which could be manifested in increased parasitism. Thus, Yevich and Barszcz (1983) noted that mussels, *M. edulis*, from the northeast coast of the United States were more extensively and intensively parasitized (three to five species of unidentified parasites) than were those from other areas. The authors pointed out that the increased parasitism occurred in waters near centers of human population density and industrial activity. In a related study of the effects of chronic exposure to various crude oils on pond-held oysters, Barszcz *et al.* (1978) found a similar increase in the prevalence and intensity of parasites—with the conclusion that resistance to infection can be reduced in polluted environments, increasing susceptibility to some parasites.

VII. CONCLUSIONS

In considering pollution-associated diseases of shellfish, a number of conclusions seem warranted:

1. Environmental stress from pollutants seems to be an important determining factor in several shellfish diseases. Effects include direct chemical damage to membranes or tissues, modification of physiological and biochemical reactions, increased infection pressure from facultative microbial pathogens, and reduced resistance to infection.

2. The presence of marginal or degraded estuarine/coastal environments may be signaled by the appearance, or the increase in prevalence, of several diseases, including shell disease in crustaceans and certain neoplasms in bivalve molluscs, but an absolute cause-and-effect relationship has not yet been demonstrated for most of these diseases.
3. The multifactorial genesis of disease in marine species is becoming apparent, involving environmental stress, facultative pathogens, resistance of hosts, and latent infections.
4. A number of viruses have been found in crustaceans and in molluscs in recent years, and the pathogenic role of two of them (shrimp *Baculovirus* and oyster *Herpesvirus*) has been demonstrated by exposure to increasing environmental stress. Other latent viral infections of invertebrates may be identified by similar experimental methods.
5. Among the most severe and persistent problems in establishing pollutant-disease relationships are the absence of baseline information about the organisms and their habitats prior to pollution, the existence of multiple pollutants in many badly degraded habitats, and the circumstantial nature of much of the evidence linking pollution and disease.
6. Experimental exposure of crustaceans to toxic chemicals has provided data useful to understanding defense mechanisms—especially the role of the black gill syndrome in sequestering and then removing toxic substances and pathogens.

In concluding this chapter, it might be relevant to summarize opinions on the subject of pollution-associated diseases of shellfish published recently by the International Council for the Exploration of the Sea's Working Group on Pathology and Diseases of Marine Organisms (1985). Principal conclusions were:

1. An established link between diseases and pollution does not exist.
2. While environmental factors have a role in the etiology of at least some diseases, most diseases have a multifactorial etiology.
3. Environmental contaminants do not always have the same effect on different species; even when chemicals may cause anomalies in one marine population, in another population exposed to the same chemicals, anomalies may not be observed.
4. It may be possible to use certain pathological signs as indicators of general water quality deterioration.

5. The most likely general effect of pollution is a nonspecific lowering of resistance, resulting in the appearance of variable disease signs rather than the specific induction of an identifiable syndrome.

These opinions seem unnecessarily pessimistic and negative, in view of the information summarized in this chapter. Whereas it is certainly true that some of the evidence is circumstantial and that more data are needed, it seems nonetheless that some measure of association between certain shellfish diseases and pollution has been demonstrated. Studies using a combination of field observations and experimental exposures to contaminants could do much to further reduce uncertainties.

Despite the relative shallowness of the data base, it is becoming apparent that many of the disorders of molluscs and crustaceans in degraded habitats are stress related and are often expressions of a stress syndrome, quite distinct from that of fish, yet similar in that heterostasis—a heightened physiological response to continuing stress—is achieved.

REFERENCES

- Aiken, D. E., and Byard, E. H. (1972). Histological changes in lobsters (*Homarus americanus*) exposed to yellow phosphorus. *Science* **176**, 1434–1435.
- Alzieu, C., Héral, M., Thibaud, Y., Dardignac, M. J., and Feuillet, M. (1982). Influence des peintures antisalissures à base d'organostanniques sur la calcification de la coquille de l'huître *Crassostrea gigas*. *Rev. Trav. Inst. Pêches Marit.* **44**, 301–349.
- Alzieu, C., Sairjuan, J., Deltreil, J. -P., and Borel, M. (1986). Tin contamination in Arcachon Bay: Effects on oyster shell anomalies. *Mar. Pollut. Bull.* **17**, 494–498.
- Ames, B. N. (1972). A bacterial system for detecting mutagens and carcinogens. In "Mutagenic Effects of Environmental Contaminants" (H. E. Sutton and M. I. Harris, eds.), pp. 57–66. Academic Press, New York.
- Anderson, R. S. (1980). Hemolysins and hemagglutinins in the coelomic fluid of a polychaete annelid, *Glycera dibranchiata*. *Biol. Bull. (Woods Hole, Mass.)* **159**, 259–268.
- Anderson, R. S. (1981). Inducible hemolytic activity in *Mercenaria mercenaria* hemolymph. *Dev. Comp. Immunol.* **5**, 575–585.
- Anderson, R. S., and Chain, B. M. (1982). Antibacterial activity in the coelomic fluid of a marine annelid, *Glycera dibranchiata*. *J. Invertebr. Pathol.* **40**, 320–326.
- Anderson, R. S., Giam, C. S., Ray, L. E., and Tripp, M. R. (1981). Effects of environmental pollutants on immunological competency of the clam *Mercenaria mercenaria*: Impaired bacterial clearance. *Aquat. Toxicol.* **1**, 187–195.
- Anderson, R. S., Giam, C. S., and Ray, L. E. (1984). Effects of hexachlorobenzene and pentachlorophenol on cellular and humoral immune parameters in *Glycera dibranchiata*. *Mar. Environ. Res.* **14**, 317–326.
- Appeldoorn, R. S., and Oprandy, J. J. (1980). Tumors in soft-shell clams and the role played by a virus. *Maritimes* **24**(1), 4–6.
- Arimoto, R., and Tripp, M. R. (1977). Characterization of a bacterial agglutinin in the hemolymph of the hard clam, *Mercenaria mercenaria*. *J. Invertebr. Pathol.* **30**, 406–413.

- Barry, M. M., and Yevich, P. P. (1972). Incidence of gonadal cancer in the quahaug (*Mercenaria mercenaria*). *Oncology* **26**, 87–96.
- Barry, M. M., and Yevich, P. P. (1975). The ecological, chemical and histopathological evaluation of an oil spill site. Part III. Histopathological studies. *Mar. Pollut. Bull.* **6**, 171–173.
- Barry, M. M., Yevich, P. P., and Thayer, N. H. (1971). Atypical hyperplasia in the soft-shell clam *Mya arenaria*. *J. Invertebr. Pathol.* **17**, 17–27.
- Barszcz, C. A., Yevich, P. P., Brown, L. R., Yarbrough, J. D., and Minchew, C. D. (1978). Chronic effects of three crude oils on oysters suspended in estuarine ponds. *J. Environ. Pathol. Toxicol.* **1**, 879–895.
- Bayne, B. L. (1975). Aspects of physiological condition in *Mytilus edulis* L., with special reference to the effects of oxygen tension and salinity. *Proc. Eur. Mar. Biol. Symp.*, 9th 213–238.
- Bayne, B. L., and Scullard, C. (1977). Rates of nitrogen excretion by species of *Mytilus* (Bivalvia: Mollusca). *J. Mar. Biol. Assoc. U.K.* **57**, 355–369.
- Bayne, B. L., Holland, D. L., Moore, M. N., Lowe, D. M., and Widdows, J. (1978). Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. *J. Mar. Biol. Assoc. U.K.* **58**, 825–841.
- Bayne, B. L., Moore, M. N., Widdows, J., Livingstone, D. R., and Salkeld, P. (1979). Measurement of the response of individuals to environmental stress and pollution: Studies with bivalve molluscs. *Philos. Trans. R. Soc. London, Ser. B* **286**, 563–581.
- Berthou, F., Balouet, G., Bodennec, G., and Marchand, M. (1987). The occurrence of hydrocarbons and histopathological abnormalities in oysters for seven years following the wreck of the *Amoco Cadiz* in Brittany (France). *Mar. Environ. Res.* **23**, 103–133.
- Brown, R. S. (1980). The value of the multidisciplinary approach to research on marine pollution effects as evidenced in a three-year study to determine the etiology and pathogenesis of neoplasia in the soft-shell clam, *Mya arenaria*. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **179**, 125–128.
- Brown, R. S., Wolke, R. E., and Saila, S. B. (1976). A preliminary report on neoplasia in feral populations of the soft-shell clam, *Mya arenaria*: Prevalence, histopathology and diagnosis. *Proc. Int. Colloq. Invertebr. Pathol.*, 1st pp. 151–159.
- Brown, R. S., Wolke, R. E., Saila, S. B., and Brown, C. W. (1977). Prevalence of neoplasia in 10 New England populations of the soft-shell clam (*Mya arenaria*). *Ann. N.Y. Acad. Sci.* **298**, 522–534.
- Brown, R. S., Wolke, R. E., Brown, C. W., and Saila, S. B. (1979). Hydrocarbon pollution and the prevalence of neoplasia in New England soft-shell clams (*Mya arenaria*). In "Animals as Monitors of Environmental Pollutants," pp. 41–51. Natl. Acad. Sci., Washington, D.C.
- Calabrese, A., Collier, R. S., Nelson, D. A., and MacInnes, J. R. (1973). The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. *Mar. Biol. (Berlin)* **18**, 162–166.
- Cheng, T. C., and Sullivan, J. T. (1984). Effects of heavy metals on phagocytosis by molluscan hemocytes. *Mar. Environ. Res.* **14**, 305–315.
- Christensen, D. J., Farley, C. A., and Kern, F. G. (1974). Epizootic neoplasms in the clam *Macoma balthica* (L.) from Chesapeake Bay. *J. Natl. Cancer Inst.* **52**, 1739–1749.
- Clark, R. C., Jr., Finley, J. S., and Gibson, G. G. (1974). Acute effects of outboard motor effluent on two marine shellfish. *Environ. Sci. Technol.* **8**, 1009–1014.
- Corner, E. D. S. and Cowey, C. B. (1968). Biochemical studies on the production of marine zooplankton. *Biol. Rev.* **43**, 393–426.
- Couch, J. A. (1974a). Free and occluded virus, similar to *Baculovirus*, in hepatopancreas of pink shrimp. *Nature (London)* **247**, 229–231.

- Couch, J. A. (1974b). An enzootic nuclear polyhedrosis virus of pink shrimp: Ultrastructure, prevalence, and enhancement. *J. Invertebr. Pathol.* **24**, 311-331.
- Couch, J. A. (1976). Attempts to increase *Baculovirus* prevalence in shrimp by chemical exposure. *Prog. Exp. Tumor Res.* **20**, 304-314.
- Couch, J. A. (1977). Ultrastructural study of lesions in gills of a marine shrimp exposed to cadmium. *J. Invertebr. Pathol.* **29**, 267-288.
- Couch, J. A. (1978). Diseases, parasites, and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North America. *Fish. Bull.* **76**, 1-44.
- Couch, J. A., and Courtney, L. (1977). Interaction of chemical pollutants and virus in a crustacean: A novel bioassay system. *Ann. N.Y. Acad. Sci.* **298**, 497-504.
- Couch, J. A., and Nimmo, D. R. (1974a). Ultrastructural studies of shrimp exposed to the pollutant chemical, polychlorinated biphenyl (Aroclor 1254). *Bull. Pharmacol. Environ. Pathol.* **11**, 17-20.
- Couch, J. A., and Nimmo, D. R. (1974b). Detection of interactions between natural pathogens and pollutants in aquatic animals. *Louisiana State Univ. Cent. Wetland Resour. Publ. LSU-SG-74-05*, 261-268.
- Couch, J., Gardner, G., Harshbarger, J. C., Tripp, M. R., and Yevich, P. P. (1974). Histological and physiological evaluations in some marine fauna. In "Marine Bioassays Workshop Proceedings 1974," pp. 156-173. Mar. Technol. Soc., Washington, D.C.
- Couch, J. A., Courtney, L. A., Winstead, J. T., and Foss, S. S. (1979). The American oyster (*Crassostrea virginica*) as an indicator of carcinogens in the aquatic environment. In "Animals as Monitors of Environmental Pollutants," pp. 65-84. Nat. Acad. Sci., Washington, D.C.
- Dawe, C. J. (1969). Phylogeny and oncogeny. *Natl. Cancer Inst. Monogr.* **31**, 1-39.
- Dawe, C. J., and Harshbarger, J. C. (Eds.) (1969). "Neoplasms and Related Disorders of Invertebrate and Lower Vertebrate Animals." *Natl. Cancer Inst. Monogr.* **31**, 772 p.
- Dixon, D. R. (1983). Sister chromatid exchange and mutagens in the aquatic environment. *Mar. Pollut. Bull.* **14**, 282-284.
- Dixon, D. R., and Clarke, K. R. (1982). Sister chromatid exchange, a sensitive method for detecting damage caused by exposure to environmental mutagens in the chromosomes of adult *Mytilus edulis*. *Mar. Biol. Lett.* **3**, 163-172.
- Dixon, D. R., and Pollard, D. (1985). Embryo abnormalities in the periwinkle, *Littorina "saxatilis"*, as indicators of stress in polluted marine environments. *Mar. Pollut. Bull.* **16**, 29-33.
- Doughtie, D. G., and Rao, K. (1983a). Ultrastructural and histological study of degenerative changes leading to black gills in grass shrimp exposed to a dithiocarbamate biocide. *J. Invertebr. Pathol.* **41**, 33-50.
- Doughtie, D. G., and Rao, K. (1983b). Ultrastructural and histological study of degenerative changes in the antennal glands, hepatopancreas, and midgut of grass shrimp exposed to two dithiocarbamate biocides. *J. Invertebr. Pathol.* **41**, 281-300.
- Dow, R. L. (1975). Reduced growth and survival of clams transplanted to an oil spill site. *Mar. Pollut. Bull.* **6**, 124-125.
- Estrella, B. T. (1984). Black gill and shell disease in American lobster (*Homarus americanus*) as indicators of pollution in Massachusetts Bay and Buzzards Bay, Massachusetts. *Mass. Dep. Fish. Wildl. Rec. Veh., Div. Mar. Fish. Publ.* **14049-19-125-5-85-C.R.**, 17 pp.
- Evans, J. E. (1978). Feasibility of using bacterial strains (mutagenesis) to test for environmental carcinogens. *U.S. Environ. Prot. Agency, Doc.* **EPA-600/3-78-042**, 118 pp.
- Farley, C. A. (1969a). Sarcomatoid proliferative disease in a wild population of blue mussels (*Mytilus edulis*). *J. Natl. Cancer Inst.* **43**, 509-516.
- Farley, C. A. (1969b). Probable neoplastic disease of the hematopoietic system in oysters, *Crassostrea virginica* and *Crassostrea gigas*. *Natl. Cancer Inst. Monogr.* **31**, 541-555.

- Farley, C. A. (1976). Proliferative disorders in bivalve mollusks. *Mar. Fish. Rev.* **38**, 30–33.
- Farley, C. A., and Sparks, A. K. (1970). Proliferative diseases of hemocytes, endothelial cells, and connective tissue cells in mollusks. *Bibl. Haematol.* **36**, 610–617.
- Farley, C. A., Banfield, W. G., Kasnic, G., Jr., and Foster, W. S. (1972). Oyster herpes-type virus. *Science* **178**, 759–760.
- Feng, S. Y., Khairallah, E. A., and Canzonier, W. J. (1970). Hemolymph free amino acids and related nitrogenous compounds of *Crassostrea virginica* infected with *Bucephalus* sp. and *Minchinia nelsoni*. *Comp. Biochem. Physiol.* **34**, 547–556.
- Frierman, E. M. (1976). Occurrence of hematopoietic neoplasms in Virginia oysters (*Crassostrea virginica*). *Mar. Fish. Rev.* **38**(10), 34–36.
- Fries, C., and Tripp, M. R. (1976). Effects of phenol on clams. *Mar. Fish. Rev.* **38**(10), 10–11.
- Fries, C. R., and Tripp, M. R. (1980). Depression of phagocytosis in *Mercenaria* following chemical stress. *Dev. Comp. Immunol.* **4**, 233–244.
- Gardner, G. R., Yevich, P. P., and Rogerson, P. F. (1975). Morphological anomalies in adult oyster, scallop, and Atlantic silversides exposed to waste motor oil. In "Proceedings of the 1975 Conference: Prevention and Control of Oil Pollution, San Francisco, California," pp. 473–477. Am. Pet. Inst., New York.
- Gopalan, U. K., and Young, J. S. (1975). Incidence of shell disease in shrimps in the New York Bight. *Mar. Pollut. Bull.* **6**, 149–153.
- Huberman, E. (1975). Mammalian cell transformation and cell-mediated mutagenesis by carcinogenic polycyclic hydrocarbons. *Mutat. Res.* **29**, 285–291.
- International Council for the Exploration of the Sea, Working Group on Pathology and Diseases of Marine Organisms (1985). Report of the study group on biological effects techniques. *Int. Counc. Explor. Sea, Doc. C.M.1985/E:48*, 46 pp.
- Jeffries, H. P. (1972). A stress syndrome in the hard clam, *Mercenaria mercenaria*. *J. Invertebr. Pathol.* **20**, 242–251.
- LaRoche, G. (1972). Biological effects of short-term exposures to hazardous materials. In "Proceedings of the 1972 National Conference on Hazardous Material Spills," pp. 199–206. U.S. Environ. Prot. Agency, Washington, D.C.
- Latt, S. A., Allen, J., Bloom, S., Carrano, A., Falke, E., Kram, D., Schneider, E., Schrech, R., Tice, R., Whitfield, B., and Wolff, S. (1981). Sister chromatid exchange—A report of the GENE-TOX workshop. *Mutat. Res.* **87**, 17–62.
- Lightner, D. V. (1975). Some potentially serious disease problems in the culture of penaeid shrimp in North America. *Proc. U.S.-Jpn. Nat. Resour. Prog.: Symp. Aquacult. Dis.* 1974, pp. 75–97.
- Longwell, A. C. (1975). Mutagenicity of marine pollutants as it could be affecting inshore and offshore marine fisheries. *Middle Atl. Coastal Fish. Cent., Natl. Mar. Fish. Serv., Inf. Rep.* **79**, 72 pp.
- Lowe, D. M., and Moore, M. N. (1978). Cytology and quantitative cytochemistry of a proliferative atypical hemocytic condition in *Mytilus edulis* (Bivalvia, Mollusca). *J. Natl. Cancer Inst.* **60**, 1455–1460.
- Lowe, D. M., and Moore, M. N. (1979). The cytology and occurrence of granulocytomas in mussels. *Mar. Pollut. Bull.* **10**, 137–141.
- Lowe, J. I., Wilson, P. D., Rick, A. J., and Wilson, A. J. Jr. (1971). Chronic exposure of oysters to DDT, toxaphene and parathion. *Proc. Natl. Shellfish. Assoc.* **61**, 71–79.
- Lowe, J. I., Parrish, P. R., Patrick, J. M., Jr., and Forester, J. (1972). Effects of the polychlorinated biphenyl Aroclor 1254 on the American oyster, *Crassostrea virginica*. *Mar. Biol. (Berlin)* **17**, 209–214.
- Mix, M. (1975). Proliferative characteristics of atypical cells in native oysters (*Ostrea lurida*) from Yaquina Bay, Oregon. *J. Invertebr. Pathol.* **26**, 289–298.
- Mix, M. (1976). A review of the cellular proliferative disorders of oysters (*Ostrea lurida*) from Yaquina Bay, Oregon. *Prog. Exp. Tumor Res.* **20**, 275–282.

- Mix, M. C., Pribble, H. J., Riley, R. T., and Tomasovic, S. P. (1977). Neoplastic disease in bivalve mollusks from Oregon estuaries with emphasis on research on proliferative disorders in Yaquina Bay oysters. *Ann. N.Y. Acad. Sci.* **298**, 356-373.
- Mix, M. C., Trenholm, S. R., and King, S. I. (1979). Benzo[a]pyrene body burdens and the prevalence of proliferative disorders in mussels (*Mytilus edulis*) in Oregon. In "Animals as Monitors of Environmental Pollutants," pp. 52-64. Natl. Acad. Sci., Washington, D.C.
- Moore, M. N. (1976). Cytochemical demonstration of latency of lysosomal hydrolases in digestive cells of the common mussel, *Mytilus edulis*, and changes induced by thermal stress. *Cell Tissue Res.* **175**, 279-287.
- Moore, M. N. (1977). Lysosomal responses to environmental chemicals in marine invertebrates. In "Pollutant Effects on Marine Organisms" (C. S. Giam, ed.), pp. 143-154. Heath, Lexington, Massachusetts.
- Moore, M. N. (1985). Cellular responses to pollutants. *Mar. Pollut. Bull.* **16**, 134-139.
- Nascimento, I. A., Smith, D. H., Kern, F., II, and Pereira, S. A. (1986). Pathological findings in *Crassostrea rhizophorae* from Todos os Santos Bay, Bahia, Brazil. *J. Invertebr. Pathol.* **47**, 340-349.
- Neff, J. M., and Haensly, W. E. (1982). Long-term impact of the Amoco Cadiz oil spill on oysters *Crassostrea gigas* and plaice *Pleuronectes platessa* from Aber-Benoit and Aber-Wrach, Brittany, France. In "Ecological Study of the Amoco Cadiz Oil Spill," NOAA-CNEXO Rep., pp. 269-328. Natl. Oceanic Atmos. Adm., Washington, D.C.
- Nimmo, D. R., Blackman, R. R., Wilson, A. J., Jr., and Forester, J. (1971). Toxicity and distribution of Aroclor 1254 in the pink shrimp, *Penaeus duorarum*. *Mar. Biol. (Berlin)* **11**, 191-197.
- Nimmo, D. R., Lightner, D. V., and Bahner, L. H. (1977). Effects of cadmium on the shrimps, *Penaeus duorarum*, *Palaeomonetes pugio* and *Palaeomonetes vulgaris*. In "Physiological Responses of Marine Biota to Pollutants" (F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg, eds.), pp. 131-183. Academic Press, New York.
- Oprandy, J. J., Chang, P. W., Pronovost, A. D., Cooper, K. R., Brown, R. S., and Yates, V. J. (1981). Isolation of a viral agent causing hematopoietic neoplasia in the soft-shell clam, *Mya arenaria*. *J. Invertebr. Pathol.* **38**, 45-51.
- Overstreet, R. M., and Howse, H. D. (1977). Some parasites and diseases of estuarine fishes in polluted habitats of Mississippi. *Ann. N.Y. Acad. Sci.* **298**, 427-462.
- Overstreet, R. M., and Van Devender, T. (1978). Implication of an environmentally-induced hamartoma in commercial shrimps. *J. Invertebr. Pathol.* **31**, 234-238.
- Parry, J. M., Tweats, D. J., and Al-Mossawi, M. A. (1976). Monitoring the marine environment for mutagens. *Nature (London)* **264**, 538-540.
- Pauley, G. B. (1969). A critical review of neoplasia and tumor-like lesions in mollusks. *Natl. Cancer Inst. Monogr.* **31**, 509-539.
- Payne, J. F., Kiceniuk, J., Misra, R., Fletcher, G., and Thompson, R. (1983). Sublethal effects of petroleum hydrocarbons on adult American lobsters (*Homarus americanus*). *Can. J. Fish. Aquat. Sci.* **40**, 705-717.
- Pickwell, G. V., and Steinert, S. A. (1984). Serum biochemical and cellular responses to experimental cupric ion challenge in mussels. *Mar. Environ. Res.* **14**, 245-265.
- Poder, M., and Auffret, M. (1986). Sarcomatous lesions in the cockle *Cerastoderma edule*. *Aquaculture* **56**, 1-8.
- Rosen, B. (1970). Shell disease of aquatic crustaceans. *Am. Fish. Soc., Spec. Publ.* **5**, 409-415.
- Ryder, J. A. (1887). On a tumor in oyster. *Proc. Natl. Acad. Sci. U.S.A.* **44**, 25-27.
- Sawyer, T. K. (1982). Distribution and seasonal incidence of "black gill" in the rock crab, *Cancer irroratus*. In "Ecological Stress and the New York Bight: Science and Management" (G. F. Mayer, ed.), pp. 199-211. Estuarine Res. Fed., Columbia, South Carolina.

- Sawyer, T. K., Lewis, E. J., Galasso, M., Bodammer, S., Ziskowski, J., Lear, D., O'Malley, M., and Smith, S. (1983). Black gill conditions in the rock crab *Cancer irroratus*, as indicators of ocean dumping in Atlantic coastal waters of the United States. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer.* **182**, 91–95.
- Sawyer, T. K., Lewis, E. J., Galasso, M. E., and Ziskowski, J. J. (1984). Gill fouling and parasitism in the rock crab, *Cancer irroratus* Say. *Mar. Environ. Res.* **14**, 355–369.
- Schlotfeldt, H. J. (1972). Jahreszeitliche Abhängigkeit der "Schwarzfleckenkrankheit" bei der Garnele, *Crangon crangon* L. *Ber. Dtsch. Wiss. Komm. Meeresforsch.* **22**, 397–399.
- Sindermann, C. J. (1979). Pollution-associated diseases and abnormalities of fish and shellfish: A review. *Fish. Bull.* **76**, 717–749.
- Sparks, A. K. (1972). "Invertebrate Pathology: Noncommunicable Diseases," 387 pp. Academic Press, New York.
- Sparks, A. K., and Lightner D. V. (1973). A tumor-like papilliform growth in the brown shrimp (*Penaeus aztecus*). *J. Invertebr. Pathol.* **22**, 203–212.
- Stainken, D. M. (1975). "The Effect of Oil Pollution on the Physiology of *Mya arenaria*, with Special Reference to the Effect upon Respiration and Mode of Accumulation," Ph.D. thesis, 160 pp. Rutgers University, Newark, New Jersey.
- Telford, M. (1968). The effects of stress on blood sugar composition of the lobster, *Homarus americanus*. *Can. J. Zool.* **46**, 819–826.
- Telford, M. (1974). Blood glucose in crayfish. II. Variations induced by artificial stress. *Comp. Biochem. Physiol. A* **48**, 555–560.
- Tripp, M. R., Fries, C. R., Craven, M. A., and Grier, C. E. (1984). Histopathology of *Mercenaria mercenaria* as an indicator of pollutant stress. *Mar. Environ. Res.* **14**, 521–524.
- Vaughn, B. E. (Ed.) (1973). Effects of oil and chemically dispersed oil on selected marine biota—A laboratory study. *API Publ.* **4191**, Am. Pet. Inst., Washington D.C., 105 pp.
- Waldock, M. J., and Thain, J. E. (1983). Shell thickening in *Crassostrea gigas*: Organotin antifouling or sediment induced? *Mar. Pollut. Bull.* **14**, 411–415.
- Walker, H. A., Lorda, E., and Saila, S. B. (1981). A comparison of incidence of five pathological conditions in soft shell clams *Mya arenaria* from environments with various pollution histories. *Mar. Environ. Res.* **5**, 109–123.
- Weis, J. S., Gottlieb, J., and Kwiatkowski, J. (1987). Tributyl tin retards regeneration and produces deformities of limbs in the fiddler crab, *Uca pugilator*. *Arch. Environ. Contam. Toxicol.* **16**, 321–326.
- Wildish, D. J. (1970). The toxicity of polychlorinated biphenyls (PCB) in sea water to *Gammarus oceanicus*. *Bull. Environ. Contam. Toxicol.* **5**, 202–204.
- Yevich, P. P., and Barry, M. M. (1969). Ovarian tumors in the quahog. *J. Invertebr. Pathol.* **14**, 266–267.
- Yevich, P. P., and Barszcz, C. A. (1976). Gonadal and hematopoietic neoplasms in *Mya arenaria*. *Mar. Fish. Rev.* **39**, (10), 42–43.
- Yevich, P. P., and Barszcz, C. A. (1977). Neoplasia in soft-shell clams (*Mya arenaria*) collected from oil-impacted sites. *Ann. N.Y. Acad. Sci.* **298**, 409–426.
- Yevich, P. P., and Barszcz, C. A. (1983). Histopathology as a monitor for marine pollution. Results of histopathological examinations of the animals collected for the US 1976 Mussel Watch Program. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer.* **182**, 96–102.
- Young, J. S., and Pearce, J. B. (1975). Shell disease in crabs and lobsters from New York Bight. *Mar. Pollut. Bull.* **6**, 101–105.
- Young, P. H. (1964). Some effects of sewer effluent on marine life. *Calif. Fish Game* **50**, 33–41.

15

Economic Effects of Shellfish Diseases

I. INTRODUCTION

Diseases—acute or chronic, epizootic or enzootic—can have negative economic effects on shellfish producers and consumers. The effects are varied, but they can be categorized for descriptive purposes as direct, in which a clear cause-and-effect relationship is evident, or indirect, in which a second-order relationship exists. Some of the more important elements in each category are listed below.

A. Direct Effects of Disease

Examples of direct effects include

- a. Loss to producers of that portion of the crop or population that has died
- b. Reduced yield per individual or stock as a consequence of disease-related poor growth or condition, or because animals must be harvested before they have reached larger, hence more valuable, sizes
- c. Reduced yield as a consequence of culling and rejection of abnormal individuals in the catch or in aquaculture crops
- d. Reduced demand for the product because of lowered quality and aesthetic values (e.g., the presence of worms, discoloration of the flesh, thin meats, or off-odor or -taste)
- e. Possible regulatory response of closing a producing area or a fishery, or withholding products from markets, because of known or unknown effects of a disease on humans

B. Indirect Effects of Disease

Indirect effects include the following:

- a. Loss of market position for the product, because of its absence from wholesale and retail outlets or its reduced availability to those outlets
- b. Negative effects on consumers because of use of the word "disease" in news media, with persistent (even if unfounded) concern about possible effects on public health
- c. Lost or reduced crops affecting the local economy, with lost revenues, reduction in profits, unemployment, and lost purchasing power (Strand and Lipton, 1986)
- d. Cost of research and management activities aimed at mitigating the effects of disease
- e. Reduced attractiveness of the affected shellfish species to recreational fishermen, with consequent losses to coastal service industries (e.g., marinas, lodging facilities, and equipment dealers)

A detailed economic analysis is obviously beyond the needs of this volume (and the competence of the author) but some treatment of financial impacts is necessary, since disease effects must eventually be assessed in these terms.

II. EFFECTS OF DISEASE ON PRODUCERS AND CONSUMERS

A decrease in survival and/or a reduction in reproductive potential are probably the most significant effects of disease on shellfish populations. These effects are on the *supply* side of the market—the production and harvesting sector—together with factors of reduced growth rates or weight loss by diseased individuals. When faced with diseases which cause actual or potential economic losses, producers must, of course, respond vigorously if the industry is to remain viable. Some responses to disease include changes in management and harvesting strategies, such as:

- Reduction in size/age of marketing (e.g., reduced oyster size limit in epizootic areas of the pathogens *Haplosporidium nelsoni* and *Perkinsus marinus*)
- Planting in areas less subject to disease and mortalities (e.g., low-salinity areas of the Chesapeake and Delaware bays)
- Planting seed stock thinly to reduce the likelihood of disease transmission (effective with oysters in *P. marinus* outbreaks)

- Removing all oysters from a diseased area and causing it to remain unproductive for several years, thereby eliminating the pathogen population (successful in eliminating the protozoan pathogen *Bonamia ostreae* from some oyster-growing areas in Denmark and The Netherlands)
- Replacement of a susceptible stock with a more resistant one, as was done on the north coast of New Brunswick, Canada, in the late 1950s (stocks were augmented by disease-resistant oysters of the same species from Prince Edward Island)
- Replacement of a susceptible species by a more resistant species (successful in France, where stocks of *Crassostrea angulata* which were susceptible to a viral gill disease were replaced by mass imports of a more resistant species, *Crassostrea gigas*, from Pacific waters)
- Fishermen shifting to alternate species during epizootics (shifts may be temporary or permanent)

Effects of disease on the *consumption* side of the market are many, beginning with a reduced demand for products that look, taste, or smell abnormal—with subsequent disinterest in related products. This can be accompanied by a decline in local fishing economies, with unemployment and financial hardship, especially for subsistence fishermen.

Consumers are affected in a variety of ways. The reduced availability of particular products often results in higher prices, which often motivate the search for less expensive substitute foods—except for specialty items such as lobsters, for which substitution is difficult (Strand and Lipton, 1986).

Other than the deterrent effects of higher prices, changes in color, texture, taste, odor, or shape—in the *quality* of the product—can also influence consumers not to buy diseased fishery products: blue crabs with meat liberally speckled with conspicuous “pepper spots” caused by larval trematode invasion and protozoan hyperparasitization (Perkins, 1971), surf clams with blackened larval nematodes (*Sulcascaris sulcata*) in their flesh (Perkins *et al.*, 1975; Sawyer, *et al.*, 1983), tanner crabs with bitter-tasting meat as a consequence of dinoflagellate infection (Meyers *et al.*, 1987), oysters with thin, yellowish meats resulting from heavy infections of the protozoan *H. nelsoni* (Sindermann and Rosenfield, 1967), shrimp with tails heavily invaded by microsporean parasites (Sindermann, 1977), lobsters with badly eroded carapaces as a consequence of shell disease (Fisher, 1988)—all effects that reduce market demand and often require culling and discard of diseased individuals, thereby increasing the costs of production.

In addition to these reasonably obvious effects of shellfish diseases on consumer behavior, the pervasive impact of public health concerns on the marketing of seafood products must not be underestimated. Many of the concerns involve *environmental* hazards—chemical toxins such as paralytic shellfish poisoning, polychlorinated biphenyls (PCBs), or heavy metals—but some involve infectious agents, particularly viruses and bacteria in molluscan shellfish (e.g., outbreaks of typhoid, hepatitis, cholera, and gastroenteritis). Recent reports of food-borne disease outbreaks in the U.S. population disclosed that 9% were attributed to fishery products (as compared with 8% for red meat and 4% for poultry)—despite the fact that per-capita consumption of fishery products was only 7.2 kg, versus 81.3 kg for red meat and 29.7 kg for poultry (Garrett, 1986).

It is important to note, though, that some of these food-borne outbreaks attributed to fishery products may also be caused by *process-induced* hazards, such as the development of toxins in food contaminated with *Clostridium botulinum*, or *food service* hazards, such as the possibility of cross-contamination in the kitchen with microorganisms from other food products that are not cooked (Fields, 1978).

Clearly, shellfish-related public health problems exist, but often these are lumped together, in the public perception, with epizootic diseases that affect shellfish but do not infect humans. It is such perceptions that create a negative influence on markets. This is not to argue that animal and human health considerations should not be interwoven in any consumer protection plan, as was pointed out by Garrett (1986). As a good example, land-based animal production (i.e., red meat and poultry) is surrounded by an elaborate U.S. Department of Agriculture/state consumer protection program, in which animal health is an important ingredient (since a number of diseases of warm-blooded animals are transmissible to humans and since diseases reduce the efficiency of animal production systems). A counterpart program does not exist for fish and shellfish, although the Food and Drug Administration and the states exercise some regulatory control (Garrett, 1986). Reasons for the discrepancy are complex, and are based in part on the continuing common-property aspect of much of seafood production, as well as the haphazard evolution of consumer protection measures for fishery products. It is impossible, in the case of wild marine populations, to achieve the same level of animal disease control that has been achieved in terrestrial animal production, so this important element of a total consumer protection program is absent. What remains is the regulatory element, which is at present narrowly oriented toward human health interests, but virtually ignores aquatic animal health. A better balance could be

achieved; the development of aquaculture production could well provide the impetus needed, since the control of animal diseases and the assurance of safe products can both be of critical importance to long-term economic viability of aquaculture ventures.

III. LOSSES IN SHELLFISH AQUACULTURE

Economic losses due to diseases are especially apparent and even quantifiable in aquaculture operations. Major elements are disease-caused reductions in stock and the cost of disease control measures.

Accumulated evidence from oyster, shrimp, and salmon aquaculture demonstrates that disease-caused mortalities and the necessity of disease control measures are significant factors in evaluating the profitability of any venture (Sindermann and Lightner, 1988). Some data exist, particularly for freshwater species, about the costs of disease control. In U.S. federal salmonid fish hatcheries, for example, disease control costs have been estimated at 10–15% of the total production costs, while several state hatcheries have estimated such costs at 20–30%. As another example, mortalities due to disease and the necessity for disease control measures have been estimated at 20–30% of the commercial production costs for shrimp.

Control techniques which increase production costs include:

- Disinfection of water by ultraviolet radiation, ozonization, or chlorination
- Disinfection of holding facilities and equipment
- Chemotherapeutic agents
- Inspection and certification of eggs or seed transferred from one geographic area to another

Some efforts have been made to look at the effects of disease in shellfish aquaculture from an economic point of view. Several particularly informative reports have been published about oyster diseases and their effects.

An epizootic disease of American oysters, *Crassostrea virginica* on the east coast of the United States, caused by the protozoan *Haplosporidium nelsoni*, has been studied since its inception in 1957 in Delaware Bay and its subsequent appearance in Chesapeake Bay in 1959. University, state, and federal research on the disease and its effects has persisted throughout the 30 intervening years, fluctuating, of course, depending on funding availability. Data on prevalences and levels of mortality in the two most heavily damaged producing areas—the Dela-

ware and Chesapeake bays—have been collected, analyzed, and published (Andrews, 1966, 1968; Andrews and Frierman, 1974; Farley, 1975; Ford and Haskin, 1982). Disease-caused mortalities in existing populations in lower Delaware Bay and lower Chesapeake Bay were estimated to be in excess of 95% during the early epizootic years. Losses in the years since then fluctuated, in part as a consequence of salinity variations (particularly in the Maryland portion of Chesapeake Bay).

Long-term studies of the disease and related mortalities led to the conclusion that “at least half of all oyster deaths in lower Delaware Bay since the early 1960s have been related to MSX [*H. nelsoni*]” (Ford and Haskin, 1982; Haskin and Ford, 1983). This estimate took into account the annual variations in prevalence, and even disclosed a possible cyclic activity of 6–8 years for the pathogen. Annual mortalities (1960–1980) ranged from less than 25% (in years of low activity) to a peak of 67% in 1979.

Reported mortality rates due to *H. nelsoni* in Chesapeake Bay are drawn from shorter data sets; oysters in the Virginia portion of the Bay experienced annual mortality rates greater than 50% in the mid-1960s (Andrews, 1968), and in the Maryland part of the Bay mortality rates varied from 2 to 55% during 1961–1968 (Farley, 1975).

In one of the few attempts to relate disease effects to monetary values, Sieling (1971) examined lost revenue from oyster production in Chesapeake Bay, due to *H. nelsoni* disease, and found that the losses were offset by increases in dockside prices—so that the fisherman's income remained relatively stable, or in some instances actually increased. Average annual production in Chesapeake Bay during the initial epizootic period (1960–1969) was only *half* that of the 15-year preceding period (1945–1959), but prices *doubled* as stocks became scarce (Sieling, 1971). The situation in Delaware Bay during the initial epizootic period (1958–1967) was not as bright, despite a similar doubling of dockside prices, since average annual production dropped to only 10% of preepizootic levels. Lost revenues in that Bay were estimated at \$3 million per year for the 12-year period. Assuming a retail market value of four to five times the dockside value, Sieling estimated, conservatively, that losses due to *H. nelsoni* disease in Delaware Bay totaled \$120 million over the 10-year reference period after mortalities began.

A second severe pathogen of American oysters, the protozoan *Perkinsus marinus*, is especially active in the warmer, high-salinity waters of the Gulf of Mexico and the southeastern Atlantic coast (northward to Chesapeake Bay). In Louisiana the entire crop of oysters over 1 year of age was often destroyed during one summer, during an epizootic period (Ray and Chandler, 1955). Disease-caused mortality of adult oysters in

some growing areas of western Florida exceeded 50% annually during 1966–1969, and was considered “a major factor in the limitation of oyster populations in Florida growing areas” (Quick and Mackin, 1971). Deaths from other natural causes were estimated at about 15% of the adult population annually.

In more northern waters—the Virginia portion of Chesapeake Bay—*P. marinus* is limited by low temperatures in the winter, with a corresponding shorter period of pathogenicity (Andrews and Hewatt, 1957). Because of this difference, oysters may be held in growing areas for longer periods before the yield is reduced to a level which returns no profit—except during epizootic years of high temperatures and salinities. In one informative early study it was shown that oysters in areas affected by *P. marinus* disease reached maximum biomass 18–24 months after transplanting; after the second summer, growth failed to equal losses due to mortalities, and the yield declined (McHugh and Andrews, 1955). An added factor in biomass reduction—beyond mortalities—was that weights of meats of heavily infected oysters were 33% less than those of uninfected ones (Ray *et al.*, 1953). A similar dual phenomenon of death and weight loss has been noted in *H. nelsoni* infections.

Other oyster pathogens can cause significant population reductions in epizootic periods. The protozoans *Marteilia refringens* and *Bonamia ostreae* have each, in turn, threatened flat oyster (*Ostrea edulis*) production in several North European countries during the past two decades. In Brittany, France, for example, annual mortalities of 70–90% occurred during 1970–1976 due to the effects of *M. refringens*, and annual mortalities of 80–90% occurred during 1980–1982 due to the effects of *B. ostreae* (Grizel, 1983, 1985). Revenues from flat oyster culture in Brittany had declined by 50% at the time of Grizel's 1983 report, and have declined further since then, even though offsetting production of the introduced (and resistant) Pacific oyster, *Crassostrea gigas*, has increased from zero to more than 100,000 tons during the same period. Meuriot and Grizel (1984) estimated that the two protozoan diseases reduced flat oyster production in 1974–1982 sufficiently to cause revenue losses of 200 million francs per year, for a total of 1.8 billion francs. They noted, for comparative purposes, that the direct and indirect losses to the oyster industry due to the much-publicized *Amoco Cadiz* oil spill were estimated at 88 million francs for 1978 (the year of the spill) (Bonnieux *et al.*, 1980, cited by Grizel, 1983). Grizel (1985), in a detailed review of both protozoan diseases, included a discussion of the economic repercussions of the mortalities during 1974–1982, in addition to the losses just mentioned. He noted, for example, that even though part of the market for the flat oyster, *O. edulis*, had been assumed by the introduced Pacific

oyster, *C. gigas*, the value and market appeal of the latter were lower, by a factor of 20% of the cost of production.

Turning to disease effects on crustaceans, there are numerous examples of severe mortalities in shrimp culture (Overstreet, 1986). Several viral diseases can affect production. Cultures of *Penaeus merguensis* and *Penaeus semisulcatus* infected by hepatopancreatic parvovirus (HPV) were reported to suffer 50–100% mortalities in 60 days, and *Penaeus stylirostris* infected with infectious hyperdermal and hematopoietic necrosis virus (IHHNV) suffered greater than 90% mortality within 2 weeks (Lightner, 1985). As Overstreet pointed out, "Time and effort spent in holding a stock that will ultimately die or be destroyed and in decontaminating the systems can account for a greater loss than the monetary value of the lost product. . . ."

Managers of shrimp aquaculture operations usually look upon a certain amount of "shrinkage" of early life history stages as "normal" and become concerned only when mortalities occur above this level. In calculating expected optimal production, losses may be estimated by some aquaculturists to be as high as 20–50% of larvae and 20% of postlarvae (Overstreet, 1986). These estimates are usually rule-of-thumb, based on experience, and they may be remarkably incorrect if disease outbreaks occur at any life stage. Disease thus introduces an uncertainty factor into such calculations of production, making the diagnosis and control of infections a critical component of culture technology. This is particularly true of diseases of juveniles, since investment (e.g., food, facilities, labor) increases markedly with advancing age of the animals.

IV. LOSSES IN NATURAL SHELLFISH POPULATIONS

Economic losses due to disease in natural populations may be accepted intuitively, but they are less quantifiable than are those in aquaculture populations. The best data (such as they are) come from studies of epizootics in coastal/estuarine shellfish populations. Almost all of these studies are descriptive, but some include attempts to provide a rough estimate of the extent of mortalities and the disease prevalences in survivors at the time of sampling. Some investigations extend over several years, but most programs decline and disappear as soon as the crisis appears to be over. Very few of the investigations include any economic impact evaluations, such as estimates of the weight or numbers of individuals eliminated from the total population and the size of that total population.

Monetary estimates of disease-related losses to producers and con-

sumers of seafood products are best made through the integrated efforts of economists, pathologists, and stock assessment biologists, since each discipline can contribute a needed perspective in determining the impacts of disease. This combination of talents has rarely, if ever, been assembled, although some partial efforts have been made (Munro *et al.*, 1983). Usually, it is the pathologist who has made the rough estimates of disease-related losses—and these have naturally been considered “too imprecise” by resource assessment biologists and “naive” by economists. A few examples of the attempts that have been made (mostly by biologists) will illustrate the distance yet to be traveled in estimating the economic impact of disease in natural shellfish populations.

Looking first at the effects of disease on natural (wild) populations of molluscs, a recent epizootic of a lethal sarcomatoid proliferative disease of soft-shell clams, *Mya arenaria*, from Chesapeake Bay, can provide useful insights. The disease first appeared in 1979 and reached outbreak proportions in late 1983, with prevalences of 40% in some samples and with an overall prevalence of 30% in the 1984 samples (Farley *et al.*, in press). Laboratory experiments demonstrated that the disease was progressive and invariably fatal (Farley *et al.*, 1986). Production of clams in Maryland waters of Chesapeake Bay during the epizootic period, 1983–1985, declined by 50% (and the price per bushel doubled). A detailed study of one producing area in 1984 and in 1985 disclosed a rapid sequence of initial high prevalences, followed by high mortalities, with consequent decreases in clam density and catch-per-unit effort. The area was considered commercially unproductive in 1986 (Farley, 1989).

Among the crustaceans, blue crabs, *Callinectes sapidus*, from the U.S. East Coast, are sometimes infected with an especially virulent amoeba, *Paramoeba pernicioso*. Mortalities of up to 30% were reported in crabs being held in shedding tanks, during an outbreak of *P. pernicioso* or “gray crab” disease in the 1960s and the early 1970s in the Middle Atlantic states (Hargis, 1985). Moribund and dead crabs were also found at coastal sampling sites from Maryland to Georgia. From concurrent field sampling and laboratory studies, an estimated 30% of crabs in Chincoteague Bay were considered to have been killed by the pathogen in a single month in 1971 (Newman and Ward, 1973). However, no long-term decline in abundance followed the outbreak.

Another crab pathogen, originally known for its effects on crab meat rather than as a lethal pathogen, is the fungus *Trichomarix invadens*, which parasitizes tanner crabs, *Chionoecetes bairdi*, from the northern Pacific coast of North America. The disease which it causes, known as black mat syndrome, causes multiple economically important changes. The meats are discolored by contamination with the black pigmented

fungus, processors reject catches that are heavily infected, and fishermen avoid areas where heavily infected crabs are common—all of which result in reduced economic value of the resource (Hibbits *et al.*, 1981). Additionally, new information indicates that the pathogen may invade living tissue, producing systemic infections which, among other kinds of damage, destroy ova and sterilize female crabs (Sparks and Hibbits, 1979; Sparks, 1982; Hargis, 1986). Growth is inhibited also, affecting the time of recruitment to the fishery, and heavy infections may be fatal, affecting the abundance of recruiting year classes. Here, then, is a pathogen which can be accused of a diversity of economically important effects on the host species, from discoloring meat to causing mortality.

Other examples could be cited—such as the effects of gaffkemia, caused by the bacterium *Aerococcus viridans* var. *homari* in lobsters, or the rhizocephalan *Loxothylacus texanus* in blue crabs—but this is enough to provide the flavor of available data on disease in wild populations (i.e., interesting, suggestive of damage, but limited in relevance to economic evaluation).

V. CONCLUSIONS

In concluding this chapter, it might be expedient to consult the writings of the few economists who have explored the subject. A recent paper by Strand and Lipton (1986) contains a number of quotable statements, such as

- “. . . the economic impacts of disease organisms are wide-ranging—from altering producer expectation to reducing demand for products”
- “. . . society allocates a substantial portion of resources each year to assure cleanliness of estuarine systems. A fair portion of that allocation is undoubtedly attributable to the presence of disease organisms in fish and shellfish” (disease organisms are defined by the authors as including parasites for which shellfish act as passive hosts and microorganisms which alter quality)
- “. . . problems of fish [and shellfish] disease are ubiquitous, significant, and possibly increasing.”

Although more economic analyses are needed to augment the information being gathered by pathologists, it is apparent that disease is important to shellfish populations and to humans, as measured by lost production and revenues and by negative effects on consumer demand. It is a little surprising, though, that so many reports on disease effects—

lethal or sublethal—refer to deleterious changes in shellfish resources, but do not take the final step to convert these findings into economic values. A few research people, such as Grizel (1983, 1985) and Haskin and Ford (1983), do take that step, but they are in the minority.

One final admonition seems important here: that of affixing blame for population decreases where it properly belongs. In the instances cited in this chapter, disease has clearly been implicated in drastic population reductions (e.g., the effects of epizootic *H. nelsoni* disease on Delaware Bay oyster stocks), but other limiting factors may be operative at the same time. To take the Delaware Bay example, a decline in oyster production there predated the epizootic, and may have resulted in part from overfishing the seed beds (Haskin and Ford, 1983). In assessing the effects of any disease outbreak, it is important to examine *all* environmental variables—as well as changes in fishing practices and market demand before, during, and after the event—before reaching a conclusion about the causes of observed changes in abundance.

REFERENCES

- Andrews, J. D. (1966). Oyster mortality studies in Virginia. V. Epizootiology of MSX, a protistan parasite of oysters. *Ecology* **47**, 19–31.
- Andrews, J. D. (1968). Oyster mortality studies in Virginia. VII. Review of epizootiology and origin of *Minchinia nelsoni*. *Proc. Natl. Shellfish. Assoc.* **58**, 23–36.
- Andrews, J. D., and Frierman, M. (1974). Epizootiology of *Minchinia nelsoni* in susceptible wild oysters in Virginia, 1959 to 1971. *J. Invertebr. Pathol.* **24**, 127–140.
- Andrews, J. D., and Hewatt, W. G. (1957). Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. *Ecol. Monogr.* **27**, 1–26.
- Bonnieux, F., Dauce, P., and Rainelli, P. (1980). Impact socio-economique de la marée noire provenant de l'Amoco Cadiz. *Rapp. Inst. Natl. Rech. Agron.* pp. 1–100.
- Farley, C. A. (1975). Epizootic and enzootic aspects of *Minchinia nelsoni* (Haplosporida) disease in Maryland oysters. *J. Protozool.* **22**, 418–427.
- Farley, C. A. (1989). Selected aspects of neoplastic progression in molluscs. In "Progressive Stages of Malignant Growth/Development. Vol. 1, Part 5: Comparative Aspects of Tumor Progression" (H. E. Kaiser, ed.). Nijhoff, New York.
- Farley, C. A., Otto, S. V., and Reinisch, C. L. (1986). New occurrence of epizootic sarcoma in Chesapeake Bay soft shell clams (*Mya arenaria*). *Fish. Bull.* **84**, 851–857.
- Farley, C. A., Plutschak, D. L., and Scott, R. F. Epizootiology and distribution of transmissible sarcoma in Maryland soft-shell clams, *Mya arenaria*, 1984–1988. *Envir. Health Persp.* (in press).
- Fields, F. R. (1978). Public health constraints on food production from the sea. In "Drugs and Food from the Sea: Myth or Reality?" (P. N. Kaul, and C. J. Sindermann, eds.), pp. 425–429. University of Oklahoma Press, Norman, Oklahoma.
- Fisher, W. S. (1988). Shell disease of lobsters. In "Disease Diagnosis and Control in North American Marine Aquaculture" (C. J. Sindermann and D. V. Lightner, eds.), pp. 236–239. Elsevier, Amsterdam.

- Ford, S. E., and Haskin, H. H. (1982). History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957–1980. *J. Invertebr. Pathol.* **40**, 118–141.
- Garrett, E. S. (1986). Role of diseases in marine fisheries management. *NOAA Tech. Rep. NMFS Circ. F/NWR-16*, 650–654 (reprinted from *Trans. North Am. Wildl. Nat. Resour. Conf.* **50**, 650–654).
- Grizel, H. (1983). Impact of *Marteilia refringens* and *Bonamia ostreae* on Breton oyster culture. *Int. Counc. Explor. Sea, Doc. C.M.1983/Gen. 9*, 30 pp.
- Grizel, H. (1985). "Étude des Récentes Epizooties de l'Huître Plate *Ostrea edulis* Linné et de leur Impact sur l'Ostréiculture Bretonne," Ph.D. thesis, 145 pp. Acad. Montpellier, Montpellier, France.
- Hargis, W. J. Jr. (1986). Quantitative effects of marine diseases on fish and shellfish populations. *NOAA Tech. Rep. NMFS Circ. F/NWR-16*, 608–640 (reprinted from *Trans. North Am. Wildl. Nat. Resour. Conf.* **50**, 608–640).
- Haskin, H. H., and Ford, S. E. (1983). Quantitative effects of MSX disease (*Haplosporidium nelsoni*) on production of the New Jersey oyster beds in Delaware Bay, USA. *Int. Counc. Explor. Sea, Doc. C.M.1983/Gen.7*, 21 pp.
- Hibbits, J. G., Hughes, G. C., and Sparks, A. K. (1981). *Trichomarix invadens* gen. et sp. nov., an ascomycete parasite of the tanner crab (*Chionoectes bairdi* Rathbun, Crustacea: Brachyura). *Can. J. Bot.* **59**, 2121–2128.
- Lightner, D. V. (1985). A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. *Proc. Int. Conf. Cult. Penaeid Prawns/Shrimps, 1st*, pp. 97–103.
- McHugh, J. L., and Andrews, J. D. (1955). Computation of oyster yields in Virginia. *Proc. Natl. Shellfish. Assoc.* **45**, 217–239.
- Meuriot, E., and Grizel, H. (1984). Note sur l'impact économique des maladies de l'huître plate en Bretagne. *Rapp. Inst. Sci. Tech. Pêches Marit.* **12**, 1–19.
- Meyers, T. R., Koenman, T. M., Botelho, C., and Short, S. (1987). Bitter crab disease: A fatal dinoflagellate infection and marketing problem for Alaskan tanner crabs *Chionoectes bairdi*. *Dis. Aquat. Org.* **3**, 195–216.
- Munro, A. L. S., McVicar, A. H., and Jones, R. (1983). The epidemiology of infectious disease in commercially important wild marine fish. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **182**, 21–32.
- Newman, M. W., and Ward, G. E., Jr. (1973). An epizootic of blue crabs, *Callinectes sapidus*, caused by *Paramoeba pernicioso*. *J. Invertebr. Pathol.* **22**, 329–334.
- Overstreet, R. M. (1986). Solving parasite-related problems in cultured Crustacea. In "Parasitology—Quo Vadit?" (M. J. Howell, ed.), *Proc. 6th Int. Congr. Parasitol.*, pp. 309–318. Aust. Acad. Sci., Canberra, Australia.
- Perkins, F. O. (1971). Sporulation in the trematode hyperparasite *Urosporidium crescens* DeTurk, 1940 (Haplosporidia: Haplosporidiidae)—An electron microscope study. *J. Parasitol.* **57**, 9–23.
- Perkins, F. O., Zwerner, D. E., and Dias, R. K. (1975). The hyperparasite *Urosporidium spisuli* sp. n. (Haplosporea) and its effects on the surf clam industry. *J. Parasitol.* **61**, 944–949.
- Quick, J. A., Jr., and Mackin, J. G. (1971). Oyster parasitism by *Labyrinthomyxa marina* in Florida. *Fla., Dep. Nat. Resour., Prof. Pap.* **13**, 55 pp.
- Ray, S. M., and Chandler, A. C. (1955). *Dermocystidium marinum*, a parasite of oysters. *Exp. Parasitol.* **4**, 172–200.
- Ray, S. M., Mackin, J. G., and Boswell, J. L. (1953). Quantitative measurement of the effect on oysters of disease caused by *Dermocystidium marinum*. *Bull. Mar. Sci. Gulf Caribb.* **3**, 6–33.

- Sawyer, T. K., Rosenfield, A., Perkins, F. O., Zwerner, D. E., Dias, R. K., Lichtenfels, J. R., Madden, P. A., Jackson, G. J., Bier, J. W., Payne, W. L., and Miller, G. C. (1983). Identification of parasitic nematode larvae, *Sulcascaris sulcata*, in the calico scallop, *Argopecten gibbus* (Linnaeus), and the surf clam, *Spisula solidissima*. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer.* **182**, 140–143.
- Sieling, F. W. (1971). Economic value of research in resources management; an exposition of direct economic benefits to management from research. *Proc. Natl. Shellfish. Assoc.* **61**, 15–19.
- Sindermann, C. J. (Ed.) (1977). "Disease Diagnosis and Control in North American Marine Aquaculture," 329 pp. Elsevier, Amsterdam.
- Sindermann, C. J., and Lightner, D. V. (Eds.) (1988). "Disease Diagnosis and Control in North American Marine Aquaculture," 2nd ed., 431 pp. Elsevier, Amsterdam.
- Sindermann, C. J., and Rosenfield, A. (1967). Principal diseases of commercially important marine bivalve mollusca and crustacea. *Fish. Bull.* **66**, 335–385.
- Sparks, A. K. (1982). The histopathology and possible role in the population dynamics of tanner crab, *Chionoecetes bairdi*, of the fungus disease (black mat syndrome) caused by *Trichomaris invadens*. *Proc. Int. Symp. Genus Chionoecetes* pp. 539–545.
- Sparks, A. K., and Hibbits, J. (1979). Black mat syndrome, an invasive mycotic disease of the tanner crab, *Chionoectes bairdi*. *J. Invertebr. Pathol.* **34**, 184–191.
- Strand, I. E., and Lipton, D. W. (1986). Disease organisms, economics and the management of fisheries. *NOAA Tech. Rep. NMFS Circ. F/NWR-16*, 655–674 (reprinted from *Trans. North Am. Wildl. Nat. Resour. Conf.* **50**, 655–674).

16

Shellfish Diseases of Public Health Significance

I. INTRODUCTION

Relatively few diseases of humans have their origin in the marine environment, but of these few, those transmitted by shellfish have justly received most of the attention from public health officials. To achieve some organization in this chapter, it is important to point out four categories of the relationships of human diseases with shellfish:

1. Shellfish may become infected with many pathogens and parasites which are *not* transmissible to humans. This category includes the vast majority of the diseases considered in this volume.
2. Shellfish may become infected with several pathogens and parasites which *are* transmissible to humans, or which may alter the shellfish host chemically, making it toxic to humans. As an example, molluscan and crustacean shellfish can become infected with *Vibrio parahaemolyticus*—some strains of which can produce enteric disorders in humans if infected animals are eaten raw or undercooked. Another example would be some of the larval trematodes which infect clams, and which may cause gastroenteric disturbances in human consumers because of the formation of toxic metabolic products in clam tissues.
3. Shellfish may become infected with organisms suspected to cause human diseases, but the reported infections are rare or postulated only on the basis of experimental infections of other mammals. Included in this category would be larval nematodes such as *Echinocephalus sinensis*, *Hysterothylacium aduncum*, and *Angyostrongylus cantonensis*.
4. Shellfish may ingest, accumulate, and transmit passively micro-

organisms and toxic substances harmful to humans but often relatively harmless to the animal. Included here would be most of the microbial pathogens of public health significance which may cause typhoid, hepatitis, and cholera, and which may occur in coastal/estuarine waters. Also included here are biotoxins (paralytic shellfish poisoning (PSP) in particular), and toxic chemicals of industrial origin (especially heavy metals, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons) (Halstead, 1971).

Items in category 1 (shellfish diseases not transmissible to humans) have been considered in Chapters 2 through 7, so they are ignored in this chapter. Items in categories 2 (shellfish pathogens with demonstrated public health significance) and 3 (shellfish pathogens with less clearly defined public health significance) contain the disease agents important to both shellfish and humans. Category 4 contains microorganisms and toxic substances found in shellfish, and transmitted to humans by them, but they rarely have deleterious effects on the shellfish and so cannot be classified as disease agents—except when toxic chemicals occur in unusual concentrations (heavy metals and chlorinated hydrocarbons, for example) (Calabrese *et al.*, 1973; Dunn and Stich, 1976). Most of the public health problems associated with consumption of shellfish are in category 4, so this category cannot be minimized.

The plan here seems logical enough: to consider first the small list of shellfish diseases known to be transmissible to humans, then to examine a few potential or suspected problem areas (larval nematodes in particular), and finally to summarize briefly the diseases of humans transmitted passively by shellfish (e.g., enteric microbial agents, biotoxins of natural origin, and toxic industrial pollutants).

II. SHELLFISH PATHOGENS WITH DEMONSTRATED PUBLIC HEALTH SIGNIFICANCE

Having spent most of this volume examining many microbial and parasitic diseases of molluscan and crustacean shellfish, it would not be surprising to find at this point that some may be transmitted to humans, thereby creating public health problems. The reality, which is probably even more surprising, is that *remarkably few pathogens of shellfish are directly transmitted to (and cause infections in) people*. Of those with a known relationship, only the ubiquitous bacterium *Vibrio parahaemolyticus* and a small array of trematodes cause disease in both shellfish and humans.

Additionally, several nematodes from shellfish are suspected to be agents of human disease, but evidence is thin, and these will be deferred to Section III of this chapter, in the interest of preserving clarity in circumscribing those pathogens which *do* play dual pathogenic roles.

A. *Vibrio parahaemolyticus*

During the past several decades increasing numbers of summer bacterial enteritis outbreaks in Japan have been traced to human ingestion of raw marine fish and invertebrates (Aiso and Matsuno, 1961; Sakazaki *et al.*, 1963). The largest outbreak, which affected 20,000 people, occurred in Niigata Prefecture in 1955; it was traced to eating cuttlefish from the Sea of Japan. Examples of the involvement of marine products in gastroenteritis outbreaks can be seen in the statistics of the Japanese Ministry of Health and Welfare. The causative organism in many cases was the halophilic bacterium, *V. parahaemolyticus*. Numerous pathogenic and nonpathogenic strains have been isolated from coastal seawater, plankton organisms, bottom mud, and the body surfaces and intestines of marine fish and shellfish. Many serotypes have been recognized, and an extensive body of Japanese literature on *V. parahaemolyticus* has accumulated (Aiso and Fujiwara, 1963; Aiso and Matsuno, 1961; Fujiwara *et al.*, 1964; Iida *et al.*, 1957; Kawashima *et al.*, 1961; Sakazaki *et al.*, 1963; Takikawa, 1958; Zen-Yoji *et al.*, 1963; Fujino *et al.*, 1974).

The Oriental custom of eating raw fish and shellfish (i.e., sushi and sashimi) has undoubtedly contributed in large part to the severity of the problem there, where 70% of all reported gastroenteritis outbreaks have been associated with *V. parahaemolyticus*. The organism was first recognized in Japan in 1951—as the cause of “shirasu food poisoning” (Fujino *et al.*, 1953). A survey of sushi cooks in Japan in 1970 disclosed 15% as asymptomatic carriers of the pathogen. During the following 20 years (1951–1971) over 1200 papers on *V. parahaemolyticus*, as well as several books, were published. The natural habitat of the organism seems to be in estuaries rather than in the open sea. The infective dose for humans is 10^6 – 10^9 organisms. *Vibrio parahaemolyticus* has a short generation time (9–11 minutes)—twice as fast as *Escherichia coli* (at about 20 minutes)—which means that infective dose levels can be reached from an original population of ten organisms in 3–4 hours.

Organisms with characteristics of *V. parahaemolyticus* have been isolated from marine sources elsewhere in the world—wherever an adequate survey has been conducted. Baross and Liston (1968) reported *V.*

parahaemolyticus in sea water, sediments, and shellfish from the Puget Sound region on the North American west coast. Probably the most interesting report concerning the pathogen is that by Krantz *et al.* (1969). *Vibrio parahaemolyticus* was isolated from moribund tank-held blue crabs, *Callinectes sapidus*, from the Chesapeake Bay region of the middle Atlantic coast of the United States. The authors suggested that the vibrio is very likely a pathogen of marine animals, and secondarily a potential human pathogen. It should be noted, however, that experimental infections of crabs were not reported, and that other pathogens have recently been described as causes of blue crab mortalities. Furthermore, *V. parahaemolyticus* has since been isolated from the hemolymph of healthy crabs (Dadisman *et al.*, 1973). Also, upon capture, the hemolymph of 91% of blue crabs contained bacteria which were primarily members of the *V. parahaemolyticus/vulnificus* and *Vibrio cholerae*-like groups (Size-more, 1986; Overstreet, 1987).

The pathogenicity of *V. parahaemolyticus* for penaeid shrimp (brown shrimp, *Penaeus aztecus*) has been demonstrated experimentally (Vanderzant *et al.*, 1970). Aquarium exposure to cultured bacterial isolates from white shrimp, *Penaeus setiferus*, resulted in behavioral changes followed by death within 3 hours, and *V. parahaemolyticus* was recovered in almost pure culture.

Vibrio parahaemolyticus and *Vibrio alginolyticus* were isolated from the hemolymph of moribund lobsters, *Homarus americanus*, being held at 20–22°C in an aquarium (Brinkley *et al.*, 1976). Pathogenicity was inferred, but not demonstrated by reinoculation; the organisms were not isolated from health lobsters in the system. The authors stated that it is likely that other factors, such as stress due to acclimation to 22°C, could predispose the animals to vibrio infections and contribute to mortality.

Outbreaks of gastroenteritis traced to *V. parahaemolyticus* have been reported from many places (see, for example, Peffers *et al.*, 1973). Part of the apparent increase in number of reports in the early 1970s may have been due to greater awareness of the problem and to improved diagnostic methods, since the organism is probably the principal cause of seafood-related diarrhea and gastroenteritis in many countries (Smith, 1971; Barrow and Miller, 1972). A random sampling made in 1976 (Craun, 1976) of just a few of literally hundreds of outbreaks included these events:

1. Two separate outbreaks occurred in 1974 on cruise ships in the Caribbean. One, which affected one third of the ship's passengers, was traced to seafood cocktails possibly contaminated by raw seawater from the ship's circulating salt water system.

The second outbreak, which involved over half of the passengers on another ship, was again traced to seafood which may have been contaminated by raw seawater during food preparation (Craun, 1975).

2. An outbreak of *V. parahaemolyticus* gastroenteritis occurred in Panama, involving 30 people who had eaten a seafood dish prepared from fresh shrimp (Kourano and Vasquez, 1975).
3. Lightly cooked cockles were implicated in an outbreak in Singapore. Four strains of *V. parahaemolyticus* were isolated from patients, and *V. parahaemolyticus* was isolated from the cockles (Foo, 1975).
4. Crabmeat cocktail served on a flight from Bangkok to London in 1972 sickened passengers and crew. *Vibrio parahaemolyticus* was suspected and was isolated from samples of raw crab claws obtained at the source, as well as from the crabmeat served on the flight (Anonymous, 1972).
5. Two outbreaks of gastroenteritis caused by *V. parahaemolyticus* occurred in Maryland following picnics featuring steamed blue crabs. Investigation revealed that the cooked crabs had been delivered by truck, with baskets of live crabs loaded (unaccountably) on top of them (Anonymous, 1972), thereby allowing bacterial contamination of the cooked animals.

An important generalization that has emerged is that *V. parahaemolyticus* can cause outbreaks even when fish and shellfish are cooked. Improper processing procedures—undercooking; use of raw seawater to wash work surfaces; allowing raw seafood to drain onto cooked products; or placing cooked seafood on surfaces where raw marine animals have been shucked, cleaned, or sliced—can lead to consumption of the pathogen by humans, with resultant gastrointestinal infections.

In addition to gastrointestinal disturbances, there have been disquieting reports of tissue infections caused by marine vibrios, including *V. parahaemolyticus*. Case histories of such marine vibrio-related infections—some of them fatal and some requiring amputation—have been described (Fernandez and Pankey, 1975), and other lesser cases, in which *V. parahaemolyticus* was isolated from infected wounds, have been reported (Porres and Fuchs, 1975). Questions arose as to whether *V. parahaemolyticus* isolated from localized tissue infections acquired from estuarine/coastal waters were enteric pathogens with an altered route of entry or whether they were “nonpathogenic” vibrios with previously unsuspected virulence. One extensive study indicated that isolates from

wound infections were clearly similar to enteric forms isolated from cases of gastrointestinal illnesses in Japan (Twedt *et al.*, 1969), and were unlike isolates from estuarine waters. [It might be noted here that a new species of vibrio, *Vibrio vulnificus*, has been described recently, and the taxon includes some organisms formerly called *V. parahaemolyticus*. It was first recognized as a human pathogen in 1976 (Hollis *et al.*, 1976; Farmer, 1980). It is highly pathogenic to humans, and can cause death, especially in individuals with preexisting liver damage, in a matter of days. Septicemias may result from ingestion of contaminated raw oysters or from infection of wounds exposed to marine environments (Blake *et al.*, 1980; Oliver, 1981; Kaysner *et al.*, 1987). *Vibrio vulnificus* has not, however, been reported to cause disease in oysters or other molluscs.]

Reports of isolations of *V. parahaemolyticus* from seafood-producing estuarine/coastal areas of the world proliferated during the 1970s. An extensive survey of the Canadian Maritime Provinces disclosed high levels in near-shore waters in the summer and autumn (Varga and Hirtle, 1975). Isolations were made from coastal waters of southeastern Alaska (Vasconcelos *et al.*, 1975) and from the coast of South Africa (Bubb, 1975). Isolations were also made from fish caught on the Italian coast (Giammanco, 1973), from Australia, from the United Kingdom, and from other locations (Battey *et al.*, 1970; Colwell *et al.*, 1973). Outbreaks of food-borne illnesses due to *V. parahaemolyticus* and traceable to shellfish have been reported by the U.S. Public Health Service since 1972. (Of course an outbreak is defined as "an incident in which as few as two people experience a similar illness after ingesting a common food.") These reports have reinforced the conclusion that *V. parahaemolyticus* is a ubiquitous inhabitant of estuarine/coastal waters and that the potential for human infection exists whenever raw or improperly handled seafood is consumed.

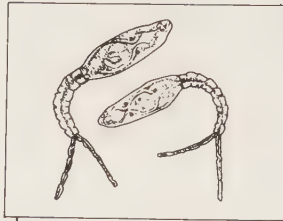
The late 1960s and the early 1970s saw the peak in research and publication on *V. parahaemolyticus* by marine microbiologists; this was followed immediately, in the late 1970s and the early 1980s, by a surge of activity with *Vibrio cholerae* from estuarine/coastal sources (DePaola, 1981). Microorganisms with characteristics of *V. cholerae* were isolated from Chesapeake Bay (Colwell *et al.*, 1977, 1981), Portugal (Ferreira and Cachola, 1975), Italy (Anonymous, 1981), and other industrialized countries. Extensive studies by Colwell and associates (Colwell *et al.*, 1981) led to the conclusion that *V. cholerae* is a component of the autochthonous flora of brackish waters, estuaries, and salt marshes of the temperate zone. Other conclusions were that *V. cholerae* can occur in the absence of fecal contamination and that sporadic outbreaks can be expected in humans when proper food-handling techniques are not

used. Sporadic outbreaks have occurred in a number of temperate zone countries—in Italy in 1973 and in 1980, in Portugal in 1974, and in Louisiana in 1978 (the first reported outbreak in the United States since 1911). Contaminated shellfish were implicated in each outbreak—mussels in Italy, cockles in Portugal, and crabs in Louisiana. Whereas *V. cholerae* may be a normal part of the brackish water microflora, its potential for causing human disease seems enhanced in heavily polluted shellfish-growing areas, especially if raw or improperly processed products are consumed or if confirmed cases of cholera have been reported in the vicinity. No reports exist of disease in shellfish caused by the organism, so further discussion of it is properly deferred to Section IV.

B. Trematode Infections

One human disease entity that has emerged in the past several decades and is related to parasitization of molluscs is marine schistosome dermatitis, often called “seabathers’ eruption” or “clam diggers’ itch.” The larval trematodes of several genera in the family Schistosomatidae responsible for this condition normally mature in the blood vessels of migratory birds. They may, however, invade human skin exposed to seawater near concentrations of the marine snails that act as intermediate hosts (Fig. 59). Penner (1942, 1950) first pointed out the existence of marine dermatitis-producing schistosomes; these have since been identified from many parts of the world (Chu, 1952; Hutton, 1952; Stunkard and Hinchliffe, 1952; Sindermann and Gibbs, 1953; Chu and Cutress, 1954; Bearup, 1956; Grodhaus and Keh, 1958; Sindermann, 1960; Ewers, 1961). Infective cercariae normally die after invading human skin, causing papular dermatitis and local inflammation, which may in some individuals be severe. Secondary bacterial infections are common among clam diggers chronically exposed to the parasites. Studies by Sindermann (1960) of *Austrobilharzia variglandis*, an avian schistosome which parasitizes the mud snail, *Nassarius obsoletus*, in New England, indicated that external environmental conditions of salinity, temperature, and oxygen influenced cercarial emergence and that infection levels varied geographically and with location of the host in the tide zone. Highest prevalences of infections—hence, greatest risk of dermatitis—occurred in the high-tide zone near bird sanctuaries in early autumn.

A new manifestation of the disease has been discovered recently on the New Jersey coast, and has been called “surfers’ itch.” Surfers seem to spend most of their time not riding waves, but belly down on surf boards, with a considerable part of their anatomy in contact with the surface film of the ocean. In areas where infective cercariae occur, ex-

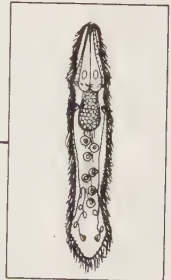


Adult *Androbelthys nasipondensis* male and female in mesenteric veins of several species of birds including LESSER SCAUP duck, BLACK duck, and RED-BREADED MERGANSER.

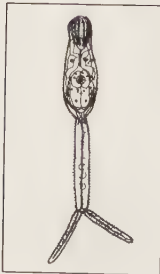


Cercaria may penetrate human skin, causing papular dermatitis and subsequent sensitization.

Cercariae usually attach to surface film where they may contact and penetrate the proper definitive host (bird) or abnormal host (human).



Miracidium hatches from egg shed in feces of bird and is free-swimming until it penetrates the proper snail intermediate host *Naasarius choleleus* in which it forms a mother sporocyst.



The cercaria is liberated from a sporocyst and emerges from the snail as a free swimming stage.



Daughter sporocysts lodge in digestive gland and gonad of the mud snail. Cercariae develop within these sporocysts.

posure in this way can produce a very interesting ring of papules across the chest, down either side of the trunk and across the thighs. Some of these fascinating findings have been published by Cohn and Blaszcak (1976).

Other quite distinct types of trematode infections may be acquired from parasitized molluscan and crustacean shellfish. A human infection with the Echinostome trematode, *Himasthla muehlensi*, was reported by Vogel (1933). The infection was apparently obtained by eating raw infected hard shell clams, *Mercenaria mercenaria*, from the east coast of the United States. Only very minor morphological differences separate *H. muehlensi* from the common clam parasite, *Himasthla quissetensis*, and it is probable that they are conspecific. Cheng (1965, 1967) has offered an interesting hypothesis to account for occasional instances of severe gastrointestinal upsets after the ingestion of raw hard clams from the New England coast. Clams from this area harbored large numbers of *H. quissetensis* metacercariae, and toxic short-chain fatty acids were found to accumulate in the clam tissues adjacent to the worms. Cheng postulated that if enough raw clams containing the toxic fatty acids were eaten, transient digestive disturbances could result. In another study Cheng (1965) also reported that the tissues of American oysters parasitized by the trematode *Bucephalus* contained appreciable amounts of short-chain fatty acids, particularly butyric acid, and again postulated that accumulations of such fatty acids in parasitized oysters could cause temporary gastroenteric disturbances in humans. In these instances, then, the public health concern about trematode parasitization of bivalves is two-fold: possible susceptibility of humans to actual infection by the worm and possible toxicity of clam tissues resulting from the presence of the metacercariae (Cheng, 1973).

A more direct association of trematodes and human disease can be found in cases of paragonimiasis in the Far East, caused by species of *Paragonimus* which infect estuarine crabs (*Sesarma dehaani* and *Sesarma intermedium*). Human lung infections can be acquired through consumption of raw or inadequately cooked crabs containing metacercariae of *Paragonimus ohirai* and *P. iloktsuensis*. The most common species of *Paragonimus*, *P. westermani*, occurs as a metacercaria in freshwater crabs, principally *Eriocheir japonicus* and *Potamon rathbuni* (Yokogawa, 1964; Cheng, 1973).

Figure 59. The life cycle of *Austrobilharzia variglandis*, an avian schistosome, and human pathology resulting from accidental invasion of the skin by cercariae. Based in part on Stunkard and Hinchliffe (1952) and Sindermann (1960).

Other trematodes of marine or estuarine origin can be public health problems. Examples would be members of the family Heterophyidae, which infect humans who eat raw mullets and other fish containing metacercariae derived from parasitized estuarine snails (Williams and Jones, 1976). A similar mollusc-to-marine/anadromous-fish-to-human cycle exists for other trematodes, such as *Heterophyes (Spelotrema) brevicaeca* from the Philippines, transmitted to humans from marine fish, and the salmon-poisoning fluke, *Nanophyetus salmincola*, from the west coast of North America, transmitted to dogs principally by salmon, but only rarely to humans. Since most trematodes have a molluscan first intermediate host, these examples should logically be considered in greater detail in this section, but since they are not transmitted *directly* from molluscs to humans (except for the avian schistosomes and some of the Echinostomes) they are not.

These, in my estimation, are the principal diseases of shellfish that may be transmitted to humans. There are other, lesser examples, but we really have to reach to consider them diseases of public health significance, in light of present knowledge; these marginal cases are considered in the next section.

III. SHELLFISH PATHOGENS WITH LESS CLEARLY DEFINED PUBLIC HEALTH SIGNIFICANCE

Humans are naturally concerned with the possibility of acquiring infections from the food they eat, including seafood. The previous section dealt with those few pathogens of shellfish able to infect people; but to complete the discussion, it is probably necessary to include the fringes—those organisms which infect shellfish and have been suspected or postulated to be agents of human disease as well. The short list considered here includes a bacterium, *Streptococcus faecalis*; a trematode, *Philophthalmus* sp.; and several nematodes (*Echinocephalus sinensis*, *Angiostrongylus cantonensis*, and *Hysterothylacium* sp.).

The ubiquitous bacterium *S. faecalis* was identified as the etiological agent for a slowly developing disease accompanied by mortalities of the shore crab, *Carcinus mediterraneus*, from the Mediterranean coast of France (Pappalardo and Boemare, 1982). The pathogen produced focal infections in hepatopancreatic connective tissue and became systemic just before death. A relationship with severe domestic sewage pollution was proposed, based on the abundance of the organism in the environment and natural selection of a variant pathogenic for crabs. Several similarities of the disease agent to *Aerococcus viridans* in lobsters were also

pointed out by the authors—especially an encapsulated diplococcus phase, similar cultural characteristics, and slow development in the host. Its histopathological manifestations were, however, quite different.

Among the parasitic worms, certain species of the trematode genus *Philophthalmus*, which occur as larvae in marine snails (Penner and Fried, 1961, 1963) and as adults in the eye membranes of gulls, may also be capable of infecting human beings. Two cases of such infection (by freshwater members of the genus) have been reported (Markovic, 1939; Dissanaik and Bilimoria, 1958) and experimental infections in mammals have been achieved (Alicata and Ching, 1960).

Several larval nematodes found in fish and shellfish are capable of penetrating the human digestive tract wall if infected hosts are consumed raw or undercooked (Young and Lowe, 1969; Ko *et al.*, 1975; Williams and Jones, 1976). The best example, of course, is human anisakiasis, caused by anisakid nematodes from marine fish. Another example would be gnathostomiasis, caused by penetration of *Gnathostoma* spp. larvae, derived from consumption of infected raw freshwater fish of the genus *Ophicephalus*. The parasites have been reported most commonly from Thailand and Japan (Takayama *et al.*, 1959; Miyazaki, 1960). Invasion of the human gut wall by the related larval gnathostome *E. sinensis* from oysters (*Crassostrea gigas*) has also been postulated, after experimental demonstration of the event in cats and in monkeys (Ko *et al.*, 1974, 1975). It seems possible, as was pointed out by Ko (1976), that human eosinophilic granulomas of unknown etiology in the Far East (Hong Kong, Singapore, China) may be consequences of invasion by larval nematodes such as *E. sinensis*.

In the same category of shellfish disease agents with a possible relationship to human diseases is the nematode *A. cantonensis*. Certain types of eosinophilic meningitis and meningoencephalitis in humans from Pacific islands are apparently caused by larval *A. cantonensis*, a nematode which normally matures in the lungs of rats (Alicata and Jindrak, 1970). Snails act as intermediate hosts and freshwater shrimp act as transport hosts of the larvae, and infection of humans can result from ingesting the raw aquatic animals (Wallace and Rosen, 1966). Migrating larvae in the meninges can produce severe and sometimes fatal disease. The role of marine animals in transmitting the larval worms is very tenuous: one marine fish, a carangid, has been experimentally infected with early larval stages of *A. cantonensis* (Wallace and Rosen, 1967), as have oysters and clams (Cheng and Burton, 1965). A history of eating raw marine fish and shellfish characterized a number of human cases investigated in Hawaii and elsewhere in the Indo-Pacific area. Intensive epidemiological and other studies have been carried on since

1960, and as research progresses, routes of infection other than those involving marine animals seem to be more important.

A possible public health risk from the consumption of unprocessed penaeid shrimp infected with larval nematodes, *Hysterothylacium* spp., was suggested by Norris and Overstreet (1976). The chain of circumstantial evidence began with reports of *Hysterothylacium* (formerly *Contracaecum*) *aduncum* in sardines off the Brittany coast of France and the possible implication of such nematodes in certain idiopathic granulomas (15 cases) in humans from the same area (Petter, 1969a,b; Benatre *et al.*, 1968; Vermeil, *et al.*, 1975). Larval *Hysterothylacium* were not demonstrated in the granulomas, and experiments with laboratory animals suggested that the larvae could not penetrate the gut wall. Nematode larvae of the genus *Hysterothylacium* (formerly *Thynnascaris*) are also common in penaeid shrimp from the Gulf of Mexico, with prevalences in several surveys of 2–80% (Norris and Overstreet, 1976). The larvae did not survive normal methods of food processing, and infectivity to experimental mammalian hosts was not demonstrated. However, *Hysterothylacium* larvae, similar to those from shrimp, but collected from the cutlassfish, *Trichiuris lepturus*, caught in the same area, were briefly infective to mice, surviving in the gut wall for several days and producing severe inflammation. The authors concluded that larval *Hysterothylacium* were less important than larval *Anisakis* as a consumer hazard, but that the potential for infection exists when raw fish or crustaceans are eaten.

It is probable that this list of suspected pathogens of shellfish and humans could be lengthened, if every association that had been speculated about or mentioned casually in the discussion section of a scientific paper were considered. But this is not necessary, especially since most of the shellfish-associated public health concerns focus on the next category of relationships to be considered: diseases of humans that are transmitted passively by shellfish.

IV. DISEASES OF HUMANS TRANSMITTED PASSIVELY BY SHELLFISH

In addition to the relatively few parasites and pathogens of shellfish which are also pathogenic to humans, there are a number of human diseases that result from contact with, or ingestion of, marine animals which carry, but are usually not themselves severely affected by, the microorganisms or toxins involved. *The function of the marine animal is usually that of concentrating or passively transporting the etiological agent*—category 4 of our original listing of human/shellfish relationships.

Such diseases are actually outside the confines of this chapter, but almost invariably, when the subject of shellfish diseases is discussed, the conversation quickly focuses on this category of public health problems, so some major candidates should probably be listed. Included would be:

1. The passive role of raw shellfish from polluted waters in transmitting microbial diseases, particularly hepatitis, typhoid, and cholera
2. The passive role of shellfish in accumulating and transmitting to humans the algal toxins that cause paralytic and other forms of shellfish poisoning
3. The role of marine animals, especially shellfish, in accumulating heavy metals and other toxic pollutants of industrial origin—to the point at which the flesh may be toxic to humans

Each of these items is clearly important from a public health perspective, so each requires a brief treatment here.

A. Microbial Diseases of Humans Transmitted by Shellfish

One large category of human diseases that are not technically derived from parasites or diseases of marine animals, but which may result from passive transfer of human pathogens by marine species, concerns viral and bacterial infections. Pollution of estuarine and coastal waters, uptake and retention of microorganisms by shellfish, and ingestion of such passive carriers, uncooked or undercooked, can unite to produce human infection (Hart, 1945; Shewan and Liston, 1954; Mason and McLean, 1962; Klein *et al.*, 1974). Examples of typhoid fever, cholera, and infectious hepatitis outbreaks traceable to the ingestion of raw shellfish can be found in the scientific literature and are often overemphasized in the newspapers. Almost invariably, when oysters or clams have been implicated, the outbreak has been traced to polluted growing areas, usually legally closed to the taking of shellfish. It seems likely that increasing conditions of estuarine and coastal pollution will lead to an increasing role of shellfish in the transfer of human pathogens. As long as raw marine products from inshore waters are eaten by humans, the possibility of disease transmission, either mechanically or as a result of multiplication of a pathogen within the marine host, must be recognized. Molluscan shellfish can be particularly dangerous in this respect. They are filter feeders, and thus can accumulate microorganisms from a polluted environment; most of them are harvested from estuaries and inshore waters, where pollution is more severe than in the open sea; and they are frequently eaten raw in otherwise nominally civilized Western

countries. Furthermore, the entire molluscan animal is usually consumed, rather than selected parts, as is the case with most fish and crustaceans.

As we dump more and more untreated or inadequately treated domestic sewage into rivers, estuaries, and coastal waters, naturally the populations of microorganisms of human origin—bacteria and viruses—in these waters will be increased. Dilution occurs, of course, as a result of tidal flushing and inshore currents, but it may not take place fast enough to remove the risk of infection as soon as necessary. Many bacteria which cause human disease do not reproduce, nor do they survive very long, in ocean waters. However, they are not killed instantaneously, and so can constitute a threat to human health. Of particular concern are microorganisms which cause cholera, typhoid, skin infections, hepatitis, botulism, and eye and ear infections. Disease-causing bacteria of human origin, present in domestic sewage, may persist for days or weeks within the digestive tracts of shellfish or on their gills, and in bottom sediments. Swimmers, skin divers, and fishermen obviously expose themselves to infection by operating near ocean outfalls. Sometimes, though, pollutants may be carried by currents for miles, so that it is difficult to determine which waters are safe and which are not.

An added element of danger results from handling or eating fish and shellfish from polluted areas. Marine animals can ingest contaminated material, and shellfish may actually accumulate viruses and bacteria (Liu *et al.*, 1966; Metcalf and Stiles, 1965). Microbial problems related to public health can be a major deterrent to full utilization of coastal resource species. Microbial diseases such as typhoid and hepatitis have been transmitted by the ingestion of raw shellfish from polluted waters (Lumsden *et al.*, 1925; Roos, 1956; Mason and McLean, 1962; Ruddy *et al.*, 1969). Viruses have been found experimentally to have variable but in some instances surprisingly long survival times in saline water and in molluscan shellfish (Metcalf and Stiles, 1966; Canzonier, 1971). Limited information is available about their viability in sludges and in bottom sediments.

One current indication of the potential for mischief by enteric viruses ingested with polluted shellfish had wire service coverage in newspapers in early 1988. The official Chinese newspaper, *China Daily*, reported a major hepatitis epidemic in Shanghai involving nearly 300,000 people. Health authorities blamed the epidemic on the consumption of clams harvested from heavily polluted coastal waters, which later proved to be contaminated with hepatitis A virus. According to the news story, about 90% of the first wave of patients had eaten the clams without thorough cooking. By mid-March 1988, the outbreak had spread to two provinces outside Shanghai.

Of the numerous viruses which may contaminate shellfish-growing areas, the so-called Norwalk virus has been implicated in recent extensive clam- and oyster-associated gastroenteritis outbreaks in the north-eastern United States (Morse *et al.*, 1986). As an example, 103 well-documented outbreaks (each involving two or more people) occurred in the state of New York in 1982, causing 1017 people to become ill. Raw oysters and raw or steamed clams were involved in the outbreaks. Clams and oysters responsible for some of the outbreaks were harvested illegally in several New England states from areas closed to fishing—suggesting a widespread pollution problem. Norwalk virus has been implicated in many shellfish-borne enteric viral outbreaks in other parts of the United States and elsewhere (Murphy *et al.*, 1979; Gunn *et al.*, 1982; Richards, 1985).

Viruses affecting humans, then, can be a critical problem for fishing or culture operations in coastal and estuarine areas where even limited domestic pollution exists, and this includes most of the areas now used or planned for use in marine aquaculture. Possible viral contamination will become an important issue where treated sludges or other fecal degradation products are used for the enrichment of growing areas, until large-scale inexpensive techniques are available which will insure viral destruction.

While the viruses constitute the most vexing public health problem in utilizing coastal species as food, pathogenic enteric bacteria form a continuing threat when raw or partially processed products are consumed by humans. Much attention has been paid to the role of a marine vibrio, *Vibrio parahaemolyticus*, in outbreaks of "food poisoning" in the Orient (and recently in the United States) related to the consumption of seafood, as discussed earlier in this chapter. While this vibrio is a normal constituent of the inshore flora, its abundance may be increased facultatively by organic enrichment of coastal and estuarine areas. Most marine bacteria are not harmful to humans, but some of the vibrios do cause digestive upsets, particularly when fish and shellfish carrying these bacteria are eaten raw or undercooked (Barker, 1974). Other pollution-associated bacteria, such as *Clostridium*, *Salmonella*, and *Shigella* should not be ignored, since a single outbreak of disease related to any marine species can have an impact on acceptance of all marine products (see, for example, Lewis, 1975).

A disturbing example of the potential danger to humans from bacterial contamination of coastal waters and of cultivated marine animals was treated extensively in the world press in the late summer of 1973. Cholera outbreaks, traced to the consumption of raw mussels from heavily polluted coastal waters, occurred in two Italian cities (Naples and Bari). The Naples outbreak killed an estimated 26 people. This scourge

from the Dark Ages has, of course, never disappeared from large areas of the planet where poor sanitation exists (especially countries such as India, Bangladesh, and Indonesia), and epidemics have occurred and are occurring in this century—but rarely in the industrialized Western nations of the temperate zone. The bacterium responsible is fragile, and is readily destroyed by normal sanitary methods. However, it can survive for weeks in seawater and can be accumulated by molluscan shellfish growing in polluted waters.

Whenever even one case of cholera occurs, the danger of shellfish contamination exists. A study in Portugal (Ferreira and Cachola, 1975) disclosed the presence of *Vibrio cholerae* in 38% of 166 samples of molluscan shellfish taken in 1974 from the vicinity of Tavira, where a case of cholera had occurred. This report was a sequel to an earlier paper (Cachola and Nunes, 1974) pointing out extensive pollution of shellfish-growing areas on the southern (Algarve) coast of Portugal. *The New York Times* (November 2, 1975) reported a subsequent cholera outbreak of over 200 cases, with three deaths in Coimbra, Portugal. Health authorities attributed the outbreak to contaminated cockles from the Mondego River estuary.

Two responses of the fishing and aquaculture industries to contamination of shellfish growing areas by enteric microbial pathogens have been "relaying" and "depuration." Some shellfish are transplanted from closed areas to relatively clean ones. This practice is reasonable if (1) the harvested shellfish from polluted beds do not inadvertently or by design end up on the market instead of in the proposed clean sites and (2) if the transplanted shellfish remain long enough in the transplant site to assure natural removal of most contaminants.

Another practice which has developed to permit utilization of shellfish from contaminated and closed beds is to require their passage through so-called depuration plants—in which the animals are placed (usually for 72 hours) in filtered ultraviolet- or ozone-treated seawater to allow natural cleansing, as determined by coliform counts. The major problems with this method are (1) the probable difference in retention time between *E. coli* and viral pathogens, (2) the likelihood that grossly polluted areas will have levels of chemical contaminants that may not respond adequately to the 72-hour treatment, and (3) the individual variability in siphoning activity of molluscan shellfish—so that some clams or oysters will be pumping clean water for only part of the 72 hours. Depuration plants possess the potential for public health problems if they are not carefully and continuously regulated (and possibly even if they are, under existing standards). We need better information about the retention time of *pathogens*, particularly viral pathogens, not just *E. coli*. We also need determinations of *chemical contaminants* in shell-

fish from polluted areas, and how levels of such contaminants are affected by depuration procedures. The biological uptake of potentially toxic heavy metals by clams and scallops has been documented (National Oceanic and Atmospheric Administration, 1976), but we need more information on retention time under depuration plant conditions by various shellfish species—not only for metals, but for such other chemicals as chlorinated hydrocarbons and petroleum residues. Only then can we have any confidence that the products of depuration are “pure” (or approach some measure of purity).

The role of shellfish in transmitting human enteric pathogens and current needs for changes in practices have been nicely summarized in an editorial in the *New England Journal of Medicine* (DuPont, 1986). Significant points were:

- “. . . microbial contamination of water and subsequently of shellfish is almost certainly becoming more common.”
- “Research laboratories should be commissioned to develop field-adaptable methods to detect hepatitis-A, Norwalk, and other similar enteric viruses.”
- “State public health authorities should strengthen the implementation and enforcement of established regulations.”
- “Consumer education should be enhanced and the public warned effectively about the hazards of eating raw or undercooked shellfish.”
- “No one should consume raw or questionably cooked shellfish in areas where hepatitis-A, cholera, or other forms of gastroenteritis are prevalent—and even during non-epidemic periods eating raw or poorly-cooked shellfish is a high-risk venture at best!”

B. Paralytic Shellfish Poisoning and Other Natural Biotoxins

Natural biotoxins in seafood have created what seem to be expanding problems—paralytic shellfish poisoning and ciguatera in particular. Seasonal toxicity to humans has characterized molluscan species from certain coastal areas (reports date back over half a century) (Sommer and Meyer, 1937), and some species of fish from particular locations in tropical and subtropical waters have long been known to be toxic. However, recent changes in the occurrence and distribution of fish and shellfish toxicity have taken place; these may be related to human modification of coastal or atoll waters. As examples, shellfish poisoning spread southward along the New England coast during the 1970s, and mussel toxicity on the northeast coast of England increased during the same period

(Ayres and Cullum, 1978; Ayres *et al.*, 1982). Other European reports of paralytic shellfish poisoning outbreaks seem to be related to high nutrient levels in coastal and harbor areas (Korringa, 1968). Recently, too, a red tide of the dinoflagellate *Gymnodinium catenatum* on the west coast of Mexico resulted in an outbreak of paralytic shellfish poisoning, with some mortalities in humans who consumed toxic oysters (*Crassostrea iridescens*) and clams (*Donax* sp.). Previous algal blooms in that area had not been toxic (Graham, 1948; Mee *et al.*, 1986). Other reports of paralytic shellfish poisoning have come from many parts of the world, including both coasts of North America, western Europe, South America, Japan, South Africa, and New Zealand (McFarren *et al.*, 1961; Hughes, 1979; Alvaria, 1979; Reyes-Vasquez *et al.*, 1979; Oshima and Yasumoto, 1979; Sullivan *et al.*, 1983; and many others).

Whatever the explanation for the observed geographic changes in the distribution and abundance of toxic organisms (and it is difficult to rule out human intrusion completely), economic and public health problems have been created. Existing toxin-monitoring programs have had to be extended and new ones created, to determine when closure of fisheries was necessary to protect seafood consumers. Especially hard hit during recent toxic episodes have been the clam industry of New England, the scallop aquaculture industry of northern Japan, and the mussel aquaculture industry of northwestern Spain. During periods of peak toxicities these industries have come to a standstill, and a "spillover" effect has been felt in marketing other seafood products.

Shellfish have been unwitting culprits in a number of outbreaks of human illnesses due to natural toxins. The list begins with the well known and widespread paralytic shellfish poisoning, due to toxic dinoflagellates of the genus *Protogonyaulax* (formerly *Gonyaulax*), and neurotoxic shellfish poisoning, caused by recurrent blooms of *Ptychodiscus brevis* (formerly *Gymnodinium breve*), which are also responsible for massive fish kills in the Gulf of Mexico (Ray and Wilson, 1957; Shimizu, 1983). Next in order of appearance is diarrhetic shellfish poisoning, due to other genera of toxic dinoflagellates (*Dinophysis*, *Prorocentrum*, and others). This poisoning was first reported in Europe in 1961 (Korringa and Roskam, 1961), became important in the late 1970s, after outbreaks in Japan and Europe, and has persisted as a significant problem in the 1980s (Kat, 1987). Then, in December 1987 a new toxin, domoic acid, was found in mussels from Prince Edward Island in the Gulf of St. Lawrence and was responsible for 129 cases of poisoning and two deaths in Canada (Pirquet, 1988). Domoic acid mimics glutamic acid, affecting the brain and the nervous system of humans. It is found in the digestive gland of

Table 19. Types of Shellfish Poisoning^a

Illness	Symptoms	Cause	Description
Paralytic shellfish poisoning	Numbness of lips, face, and extremities; visual disturbance; staggering gait; difficulty breathing; paralysis	Species of the dinoflagellate, <i>Protogonyaulax</i> (formerly <i>Gonyaulax</i>)	About 12 forms of purine-derived saxitoxins; water soluble; acts by blocking sodium channel needed to transmit nerve impulses
Neurotoxic shellfish poisoning	Nonfatal but unpleasant neurotoxic symptoms; strong action on cardiovascular system	<i>Ptychodiscus brevis</i> (formerly <i>Gymnodinium breve</i>)	Toxins have unusual polycyclic ether skeletons
Diarrhetic shellfish poisoning	Cramps; severe diarrhea; nausea, vomiting; chills; death rare	Species of dinoflagellates, <i>Dinophysis</i> and <i>Prorocentrum</i> in particular	Dinophysis toxin and okadaic acid; large, fat-soluble polyethers; can move across cell membranes and make them "leak"
Domoic acid (amnesic shellfish poisoning)	Nausea, vomiting; muscle weakness; disorientation; loss of short-term memory	Reported to be a diatom, <i>Nitzschia pungens</i> ; toxin also found in species of the red alga, <i>Chondria</i>	Nonessential amino acid; mimics glutamic acid; affects brain and nervous system; found in digestive glands of contaminated shellfish

^a Adapted from Pirquet (1988).

shellfish, especially mussels, and its origin was reported to be a persistent bloom of the diatom *Nitzschia pungens* in mussel culture areas of Prince Edward Island (Bird and Wright, 1989), although species of the red alga, *Chondria*, are also known to produce the toxin. The toxic event dealt a staggering blow to the emerging mussel aquaculture industry of the island (Waldichuk, 1988). These various forms of natural toxins are shown in Table 19.

As the reported occurrences of algal blooms and the associated phenomena of fish kills and shellfish toxicity increase, more and more research effort becomes entrained, with salutary results. Several major international conferences on the subject have been held, and the find-

ings have been published as books (LoCicero, 1975; Taylor and Seliger, 1979; Anderson *et al.*, 1985). Some recent findings are:

1. Dinoflagellate toxicity which may be poisonous to higher animals is found in at least 20 shellfish species representing at least ten genera (Mee *et al.*, 1986). Paralytic shellfish poisoning is a recurrent problem in some coastal waters of North America and Europe, and it occurs sporadically in other temperate and tropical areas.
2. Weakly toxic substances produced by *Protogonyaulax* spp. may be transformed by mild acidic extraction or by clam enzymes into saxitoxin and related toxins (Hall *et al.*, 1980; Shimizu and Yoshioka, 1981).
3. A previously unrecognized type of shellfish poisoning (diarrhetic shellfish poisoning), with diarrhea as the dominant symptom, was first seen in Europe in 1961 and was subsequently reported from several locations in Japan and in Europe (Yasumoto *et al.*, 1978, 1980; Kat *et al.*, 1982; Kat, 1987). It should be noted, though, that shellfish-associated enteric disturbances have a long history, and this type of poisoning may have been present much earlier but not diagnosed (Korringa and Roskam, 1961; Kat, 1979).
4. Resting cysts of *Protogonyaulax tamarens* from bottom sediments were found to be ten times more toxic than were motile stages. Cysts are probably ingested by shellfish, causing toxicity even when blooms are not apparent (Dale, *et al.*, 1978).
5. Markets for shellfish grown and harvested for export are especially sensitive to toxic outbreaks and may react violently. An example of this phenomenon was seen in 1976 when an epidemic of paralytic shellfish poisoning was reported in Spain, France, Italy, Germany, and Switzerland—the consequence of consumption of mussels grown in northwestern Spain (Luthy, 1979). Exports ceased for an extended period, and a monitoring system was instituted by Spain. A subsequent outbreak of diarrhetic shellfish poisoning in France affected 10,000 people in 1984 and 2000 people in 1985.
6. Blooms of algae, known previously as causes of only sporadic or localized outbreaks, seem to be occurring with increasing frequency and intensity, and more may be expected. An example would be blooms of the naked dinoflagellate *Gyrodinium aureolum*, observed to cause mortalities of marine organisms in England in 1978, in Scottish salmon cages in 1980, and in wild as

well as captive fish on the coast of Norway in 1981 and in 1982 (Ayres *et al.*, 1982; Dahl *et al.*, 1982). Another example would be mortalities of farmed salmon in Scotland in 1979 and in 1982 caused by blooms of "flagellate X," possibly a species of *Olisthodiscus* or *Chattonella*. These agents had been recognized earlier in connection with unusual blooms, but occurrences were rare and geographically restricted.

It does seem that episodes of toxicity in molluscan shellfish are increasing in geographic distribution and variety. Effects are especially critical when blooms occur in or near aquaculture operations. The possible human influence on toxic blooms is still somewhat debatable. Organic enrichment of estuarine/coastal waters will certainly enhance algal blooms and could contribute to the toxicity problem. Monitoring and surveillance systems have become a necessity as foreign markets are developed, but the control of blooms (hence, toxicity) is not yet a reality.

C. Toxic Chemicals of Industrial Origin

Fish and shellfish may accumulate dangerously high levels of pesticides, heavy metals, and other chemicals in grossly polluted waters. Of the chemicals which could occur at levels toxic to humans in seafoods, mercury has justly received the most attention. The horrors of Minamata disease, caused by mercury contamination of fish and cultivated shellfish during 1953–1961 in a bay in Japan (Sato *et al.*, 1959), was publicized on television, in news magazines, and in a book (Smith and Smith, 1975). This remains probably the most extreme example of the risks involved in eating seafood from badly contaminated waters. We have had no comparable extreme example of metal contamination of seafood in North America, nor do we have, as yet, any evidence of acute human disease resulting from the consumption of fish or shellfish with high chlorinated hydrocarbon levels. High mercury contaminant levels in swordfish in 1971 effectively eliminated a developing fishery in the Gulf of Mexico and caused a temporary ban on the sale of swordfish in the United States. Increased surveillance of this and other heavy metals in all kinds of seafood lessens the likelihood of another Minamata incident, although whenever food is grown near industrial operations there is always a risk of chemical contamination, through negligence, deliberate actions, or accidental spills. Surveillance methods cannot easily be extended to include every localized area—especially since some of the analytical methods are very time consuming and the toxic levels for some contaminants are not known, or are unstated.

Although mercury has achieved the most notoriety among heavy

metal contaminants, (Keckes and Miettinen, 1972), public health problems have been created by toxic levels of other metals as well. As an example, ingestion of cadmium-contaminated food and water causes a disease called itai-itai in Japan (Kato and Kawano, 1968).

Possible toxic effects of pesticides in foods have been discussed on many occasions. Two decades ago Butler (1969a) stated that "Despite the widespread occurrence of persistent pesticide residues in the world fauna, their magnitude is for the most part too small to have any known significant effects on human health." (Emphasis here should obviously be on the word "known".) This statement was based on the results of a monitoring program instituted in the United States in 1965 to scrutinize pesticide levels in estuarine fish and shellfish (Butler, 1969b). Pesticide residues in all samples from 15 states involved in the monitoring effort during the late 1960s and early 1970s were below levels considered hazardous to humans (Butler, 1973). Even though indiscriminate use of pesticides is coming under some measure of control in the United States, their use in other parts of the world is expanding. Because of their persistence in the environment and their accumulation by successive levels of food chains, pesticides continue to be threats in coastal/estuarine areas, including those devoted to marine aquaculture.

A number of industrial pollutants, including heavy metals and some petroleum derivatives, are carcinogenic. Their presence in the tissues of shellfish has been cause for concern, and surveys, as well as monitoring programs such as Mussel Watch, have been instituted. Thus far, there is no evidence for *direct* association of carcinogens in seawater, fish, shellfish, or bottom sediments with cancer in humans; no case of human cancer has been unequivocally traced to chemical contaminants from the marine environment. However, this does *not* imply that there is no present or future danger from contaminated marine sources.

Contaminant-related events with direct or potential public health significance seem to be increasing in frequency. While some of this may be due to increased public awareness and some improvement in surveillance, it is apparent that the geometric increase in synthetic chemical production and use in the past four decades has contributed substantially. Synthetic chemicals that simply did not exist even two decades ago are now being viewed with some alarm as environmental contaminants. Many such chemicals were dumped indiscriminately in rivers and estuaries, but increasing public environmental concerns have tended to discourage such practices. Disclosure of chemical pollution problems is still often accidental, and even after such disclosure, regulatory agencies often encounter resistance from the contaminating industries. Controls are often inadequate and action levels undetermined.

Public alarm about contamination of food and water that may affect human health can lead to closure of industries or modifications of production methods, but only after some clear evidence of danger has appeared and has been publicized in news media. There have been several recent incidents in North America that illustrate a common sequence of events: (1) release of toxic chemicals in the absence of surveillance and control and with little knowledge of their public health effects, (2) some preliminary accidental or fortuitous indication of danger, (3) vigorous denial of danger or responsibility by the industry involved, (4) entrance of regulatory agencies, and (5) often reluctant compliance by the offending industry in the presence of mounting data. Examples of this sequence include the release of PCBs in the Hudson River and the Great Lakes, the release of kepone in the James River in Virginia, and the release of phosphorus in Placentia Bay, Newfoundland.

The PCB story in the Hudson River is still unfolding. It includes deliberate and long-term release of PCBs into the upper river by two units of the General Electric Company, the finding of dangerously high levels of PCBs in fish, the closing of the river to fishing (except for certain anadromous species), and the reluctant reduction of contamination by the offending industry.

Kepone, a highly toxic and persistent insecticide, was deliberately discharged into the upper James River (an important oyster-producing area) by a subsidiary of the Allied Chemical Company. Only after obvious toxic effects on company workers were disclosed was there any concern about pollution of adjacent waters and shellfish. The producing plant was closed, and the river was closed to fishing for an extended period.

The Placentia Bay phosphorus contamination was first disclosed by the observation of red herring and extensive herring and lobster mortalities in the Bay (Aiken and Byard, 1972). A new and inefficient industrial operation had begun shortly before this and had been releasing phosphorus in the Bay (which is also a very important fishing area). The plant was closed, as was the fishery, in the presence of clear danger to public health.

So, in summary, while there is little evidence of significant direct danger to human life in existing chemical contaminant levels in marine resource species (except for very localized incidents of gross pollution such as those just described), there are many instances of contaminant levels high enough to warrant attention, further study, and possibly controlled consumption. There is also a great need for much more scientific examination of possible long-term sublethal effects of the contami-

nants in food. Enough evidence now exists about carcinogenic, mutagenic, and other long-term toxic effects of many industrial chemicals to warrant more attention and reasoned action, even in the absence of incontrovertible proof of the risk to public health.

V. CONCLUSIONS

The question "Are there diseases or parasites of shellfish that are transmissible to humans, and that thus constitute public health problems?" clearly deserves an affirmative answer, even though the most consequential public health problems are those caused by pathogenic enteric microorganisms and toxic chemicals transmitted *passively* to humans through the consumption of bivalve molluscs. To retain proper perspective, it should be clearly understood that most pathogens and parasites of shellfish are not directly infective to humans. Even those parasites which normally develop in sea birds or mammals may not mature or may be otherwise imperfectly adapted to the human host. We are left, therefore, with only a few problem areas:

- *Vibrio parahaemolyticus*
- Avian schistosomes and a few other trematodes
- Some larval nematodes of questionable pathogenicity

This short list should not be a basis for complacency, however, since so little medically oriented research has been done with marine animals.

Possible links between aquatic animal disease and human disease should not be dismissed lightly—nor should they cause panic-stimulated actions and publicity which may not be warranted by the information at hand. It is possible, for example, to achieve 100% contamination of some aquaculture stocks by a pathogen such as *V. parahaemolyticus*, which could then affect humans (with disastrous economic consequences to the industry, as well as some human misery). Many events are *possible*, but their *probability* is low if adequate precautions are taken. It is easy to extrapolate from experimental studies and from rare cases, thereby distorting the real danger to humans.

In conclusion, it seems that much of the public health significance of shellfish diseases resides not in the diseases themselves, but rather in the passive transmission by the shellfish of human pathogens and toxic chemicals. Even in the case of *V. parahaemolyticus*, which can be described as an occasional pathogen of shellfish, much of the impact is felt by human consumers of animals which may simply be transporting the infectious agent.

REFERENCES

- Aiken, D. E., and Byard, E. H. (1972). Histological changes in lobsters (*Homarus americanus*) exposed to yellow phosphorus. *Science* **176**, 1434–1435.
- Aiso, K., and Fujiwara, K. (1963). Feeding tests of "pathogenic halophilic bacteria." *Chiba Daigaku Fuhai Kenkyusho Hokoku* **15**, 34–38.
- Aiso, K., and Matsuno, M. (1961). The outbreaks of enteritis-type food poisoning due to fish in Japan and its causative bacteria. *Jpn. J. Microbiol.* **5**, 337–364.
- Alicata, J. E., and Ching, H. L. (1960). On the infection of birds and mammals with the cercaria and metacercaria of the eye-fluke, *Philophthalmus*. *J. Parasitol., Suppl.* **46**, 16 (abstract).
- Alicata, J. E., and Jindrak, K. (1970). "Angiostrongylosis in the Pacific and Southeast Asia." Thomas, Springfield, Illinois.
- Alvaria, S. (1979). Red tides off the coast of Chile. In "Developments in Marine Biology: Vol. I. Toxic Dinoflagellate Blooms". (D. L. Taylor and H. H. Seliger, eds.), pp. 161–164 Elsevier/North-Holland, New York.
- Anderson, D. M., White, A. W., and Baden, D. G. (Eds.) (1985). Toxic dinoflagellates. *Proc. Int. Conf. Toxic Dinoflagellates*, 3rd 561 pp. Elsevier/North Holland, New York.
- Anonymous (1972). *Vibrio parahaemolyticus* gastroenteritis—United Kingdom. *U.S. Public Health Serv., Morbid. Mortal. Weekly Rep.* **March 25**, 99 and 104.
- Anonymous (1981). Outbreak of *Vibrio cholerae* non-0-1 gastroenteritis—Italy. *U.S. Public Health Serv., Morbid. Mortal. Rep.* **August 7**, 374.
- Ayres, P. A., and Cullum, M. (1978). Paralytic shellfish poisoning. An account of investigations into mussel toxicity in England 1968–1977. *Ministry of Agriculture, Fisheries and Food, Direct. Fish. Res. Lowestoft, Fish. Res. Tech. Rep.* **40**, 23 pp.
- Ayres, P. A., Seaton, D. D., and Tett, P. B. (1982). Plankton blooms of economic importance to fisheries in UK waters 1968–1982. *Int. Counc. Explor. Sea, Doc. C.M.1982/L:38*, 12 pp.
- Barker, W. H., Jr. (1974). *Vibrio parahaemolyticus* outbreaks in the United States. *Lancet* **1**, 551–554.
- Baross, J., and Liston, J. (1968). Isolation of *Vibrio parahaemolyticus* from the northwest Pacific. *Nature (London)* **217**, 1263–1264.
- Barrow, G. I., and Miller, D. C. (1972). *Vibrio parahaemolyticus*: A potential pathogen from marine sources. *Lancet* **1**, 485.
- Batley, Y. M., Wallace, R. B., Allan, B. C. and Keffe, B. M. (1970). Enteritis in Australia caused by a marine vibrio. *Med. J. Aust.* **1**, 430.
- Bearup, A. J. (1956). Life cycle of *Austrotilharzia terrigalensis* Johnston, 1917. *Parasitology* **46**, 470–479.
- Benatre, A., Guntz, M., Simard, C., Lieftring, J. J., Bertrand, J., Forest, J. L., and Pelletier, G. (1968). Le granulome eosinophilique du tube digestif. *Rev. Med. Tours* **2**, 237–243.
- Bird, C. J., and Wright, J. L. C. (1989). The shellfish toxin domoic acid. *World Aquacult.* **20**, 40–41.
- Blake, P. A., Weaver, R. E., and Hollis, D. G. (1980). Diseases of humans (other than cholera) caused by vibrios. *Annu. Rev. Microbiol.* **34**, 341–367.
- Brinkley, A. W., Rommel, F. A., and Huber, T. W. (1976). The isolation of *Vibrio parahaemolyticus* and related vibrios from moribund aquarium lobsters. *Can. J. Microbiol.* **22**, 315–317.
- Bubb, R. D. (1975). *Vibrio parahaemolyticus*—A marine pathogen detected in South African coastal waters. *S. Afr. Med. J.* **49**, 1514–1517.

- Butler, P. A. (1969a). The significance of DDT residues in estuarine fauna. In "Chemical Fallout" (M. W. Miller and G. G. Berg. eds.), pp. 205-220. Thomas, Springfield, Illinois.
- Butler, P. A. (1969b). Monitoring pesticide pollution. *BioScience* **19**, 889-891.
- Butler, P. A. (1973). Residues in fish, wildlife and estuarines: Organochlorine residues in estuarine mollusks 1965-72—National Pesticide Monitoring Program. *Pestic. Monit. J.* **6**, 238-362.
- Cachola, R., and Nunes, M. C. (1974). Quelques aspects de la pollution bacteriologique des centres producteurs de mollusques de l'Algarve (1963-1972). *Bol. Inf., Inst. Biol. Marit.* **13**, 12 pp.
- Calabrese, A., Collier, R. S., Nelson, D. A., and McInnes, J. R. (1973). The toxicity of heavy metals to embryos of the American oyster, *Crassostrea virginica*. *Mar. Biol. (Berlin)* **18**, 162-166.
- Canzonier, W. J. (1971). Accumulation and elimination of coliphage S-13 by the hard clam, *Mercenaria mercenaria*. *Appl. Microbiol.* **21**, 1024-1031.
- Cheng, T. C. (1965). Histochemical observations on changes in the lipid concentration of the American oyster, *Crassostrea virginica* (Gmelin), parasitized by the trematode *Bucephalus* sp. *J. Invertebr. Pathol.* **7**, 398-407.
- Cheng, T. C. (1967). Marine mollusks as hosts for symbioses: With a review of known parasites of commercially important species. *Adv. Mar. Biol.* **5**, 1-424.
- Cheng, T. C. (1973). Human parasites transmissible by seafood—And related problems. In "Microbial Safety of Fishery Products," pp. 163-189. Academic Press, New York.
- Cheng, T. C., and Burton, R. W. (1965). The American oyster and clam as experimental intermediate hosts of *Angiostrongylus cantonensis*. *J. Parasitol.* **51**, 296.
- Chu, G. W. T. C. (1952). First report of the presence of a dermatitis-producing marine larval schistosome in Hawaii. *Science* **115**, 151-153.
- Chu, G. W. T. C., and Cutress, C. E. (1954). *Austroilharzia variglandis* (Miller and Northrup, 1926) Penner, 1953 (Trematoda: Schistosomatidae) in Hawaii with notes on its biology. *J. Parasitol.* **40**, 515-523.
- Cohn, M., and Blaszcak, D. (1976). Clam digger's itch. *Underwater Nat.* **9**(4), 17-19.
- Colwell, R. R., Lovelace, T. E., Wan, L., Kaneko, T., Staley, T., Chen, P. K., and Tubiash, H. (1973). *Vibrio parahaemolyticus*—Isolation, identification, classification and ecology. *J. Milk Food Technol.* **36**, 202-213.
- Colwell, R. R., Kaper, J., and Joseph, S. W. (1977). *Vibrio cholerae*, *Vibrio parahaemolyticus*, and other vibrios: Occurrence and distribution in Chesapeake Bay. *Science* **198**, 394-396.
- Colwell, R. R., Seidler, R. J., Kaper, J., Joseph, S. W., Garges, S., Lockman, H., Maneval, D., Bradford, H., Roberts, N., Remmers, E., Huq, I., and Huq, A. (1981). Occurrence of *Vibrio cholerae* serotype 01 in Maryland and Louisiana estuaries. *Appl. Environ. Microbiol.* **41**, 555-558.
- Craun, G. F. (1975). Microbiology—Waterborne outbreaks. *J.—Water Pollut. Control Fed.* **47**, 1566-1569.
- Craun, G. F. (1976). Microbiology—Waterborne outbreaks. *J.—Water Pollut. Control Fed.* **48**, 1378-1397.
- Dadisman, T. A., Jr., Nelson, R., Molenda, J. R., and Garber, H. J. (1973). *Vibrio parahaemolyticus* gastroenteritis in Maryland. *Am. J. Epidemiol.* **96**, 414-426.
- Dahl, E., Danielssen, D. S., and Bohl, B., (1982). Mass occurrence of *Gyrodinium aureolum* Hulburt and fish mortality along the southern coast of Norway in September-October, 1981. *Int. Counc. Explor. Sea, Doc. C.M.1982/L:56*, 9 pp.
- Dale, B., Yentsch, C. M., and Hurst, J. W. (1978). Toxicity in resting cysts of the red-tide dinoflagellate *Gonyaulax excavata* from deeper water coastal sediments. *Science* **201**, 1223-1224.

- DePaola, A. (1981) *Vibrio cholerae* in marine foods and environmental waters: A literature review. *J. Food. Sci.* **46**, 66–70.
- Dissanaike, A. S., and Bilimoria, D. P. (1958). On an infection of a human eye with *Philophthalmus* sp. in Ceylon. *J. Helminthol.* **32**, 115–118.
- Dunn, B. P., and Stich, H. F. (1976). Release of the carcinogen benzo(a)pyrene from environmentally contaminated mussels. *Bull. Environ. Contam. Toxicol.* **15**, 398–399.
- DuPont, H. L. (1986). Consumption of raw shellfish—Is the risk now unacceptable? *N. Engl. J. Med.* **314**, 707–708.
- Ewers, W. H. (1961). A new intermediate host of schistosome trematodes from New South Wales. *Nature (London)* **190**, 283–284.
- Farmer, J. J. (1980). Revival of the name *Vibrio vulnificus*. *Int. J. Syst. Bacteriol.* **30**, 656.
- Fernandez, C. R., and Pankey, G. A. (1975). Tissue invasion by unnamed marine vibrios. *JAMA, J. Am. Med. Assoc.* **233**, 1173–1175.
- Ferreira, P. S., and Cachola, R. A. (1975). *Vibrio cholerae* El Tor in shellfish beds of the south coast of Portugal. *Int. Counc. Explor. Sea, Shellfish and Benthos Comm., Doc.* **C.M.1975/K:18**, 7 pp.
- Foo, C. K. (1975). Source of *Vibrio parahaemolyticus* infection. *Singapore Med. J.* **15**, 188 (*Microbiol. Abstr.* **10**, B6866).
- Fujino, T., Okuno, Y., Nakada, D., Aoyama, A., Fukai, K., Mukai, T., and Ucho, T. (1953). On the bacteriological examination of Shirasu-food poisoning. *Med. J. Osaka Univ.* **4**, 299–304.
- Fujino, G., Sakaguchi, G., Sakazaki, R., and Takeda, Y. (1974). "International Symposium on *Vibrio parahaemolyticus*," 261 pp. Saikon Publ. Co., Tokyo.
- Fujiwara, K., Katoh, H., Tatsumi, K., Sawada, F., and Tsuchiya, Y. (1964). A selective medium for the isolation of *Vibrio parahaemolyticus* (tellurite medium-TTGA agar). *J. Food Sanit.* **5**, 211–214 (in Japanese; English summary).
- Giammanco, G. (1973). Isolamento di vibrioni alofili riferibili a *Vibrio parahaemolyticus* e *Vibrio alginolyticus* da pesci e da Campioni D'Acqua del Mediterraneo. *Boll. Ist. Sieroter. Milan.* **52**, 1–15.
- Graham, H. W. (1948). *Gymnodinium catenatum*, a new dinoflagellate from the Gulf of California. *Trans. Am. Microsc. Soc.* **62**, 259–261.
- Grodhaus, G., and Keh, B. (1958). The marine dermatitis-producing cercaria of *Austrobilharzia variglandis* in California (Trematoda: Schistosomatidae). *J. Parasitol.* **44**, 633–638.
- Gunn, R. A., Janowski, H. T., Lieb, S., Prather, E. C., and Greenberg, H. B. (1982). Norwalk virus gastroenteritis following raw oyster consumption. *Am. J. Epidemiol.* **115**, 348–351.
- Hall, S., Reichardt, P. B., and Neve, R. A. (1980). Toxins extracted from an Alaskan isolate of *Protophyaulax* sp. *Biochem. Biophys. Res. Commun.* **97**, 649–653.
- Halstead, B. W. (1971). Toxicity of marine organisms caused by pollutants. *FAO Tech. Conf. Mar. Pollut. Effects Living Resour. Fish. Doc.* **MP/70/R-6**, 21 pp.
- Hart, J. C. (1945). Typhoid fever from clams. *Conn. Health Bull.* **59**, 289–292.
- Hollis, D. G., Weaver, R. E., Baker, C., and Thornberry, C. (1976). Halophilic *Vibrio* species isolated from blood culture. *J. Clin. Microbiol.* **3**, 425–432.
- Hughes, J. M. (1979). Epidemiology of shellfish poisoning in the United States, 1971–1977. In "Developments in Marine Biology: Vol. 1. Toxic Dinoflagellate Blooms" (D. L. Taylor, and H. H. Seliger, eds.), pp. 23–28. Elsevier/North-Holland, New York.
- Hutton, R. F. (1952). Schistosome cercariae as the probable cause of seabather's eruption. *Bull. Mar. Sci. Gulf Caribb.* **2**, 346–359.
- Iida, H., Iwamoto, T., Karashimada, T., and Kumagai, M. (1957). Studies on the patho-

- genesis of fish-borne food-poisoning in summer. II. Studies on cholinesterase inhibition by culture filtrates of various bacteria. *Jpn. J. Med. Sci. Biol.* **10**, 177-185.
- Kat, M. (1979). The occurrence of *Prorocentrum* species and coincidental gastrointestinal illness of mussel consumers. In "Developments in Marine Biology: Vol. 1. Toxic Dinoflagellate Blooms" (D. L. Taylor and H. H. Seliger, eds.), pp. 215-220 Elsevier/North-Holland, New York.
- Kat, M. (1987). Diarrhetic mussel poisoning. Measures and consequences in The Netherlands. *Rapp. P.-V. Reun., Cons. Int. Explor. Mer* **187**, 83-88.
- Kat, M., Speur, J., and Otte, P. F. (1982). Diarrhetic mussel poisoning in The Netherlands related to the occurrence of *Dinophysis acuminata*, September-October 1981. *Int. Counc. Explor. Sea, Doc. C.M.1982/E:24*, 6 pp.
- Kato, T., and Kawano, S. (1968). Reviews of past and present of itai-itai disease. On the process of development. *Gendai Igaku* **16**, 29 (in Japanese).
- Kawashima, S., Hayashi, T., Watanabe, A., and Yamashita, N. (1961). Outbreaks of food poisoning caused by pathogenic halophiles and the methods for prevention. *Food Sanit. Res.* **125**, 1-71.
- Kaysner, C. A., Abeyta, C., Jr., Wekell, M. M., DePaola, A., Jr., Scott, R. F., and Leitch, J. M. (1987). Incidence of *Vibrio cholerae* from estuaries of the United States west coast. *Appl. Environ. Microbiol.* **53**, 1344-1348.
- Keckes, S., and Miettinen, J. K. (1972). Mercury as a marine pollutant. In "Marine Pollution and Sea Life" (M. Ruivo, ed.), pp. 276-289. Fish. News Books, London.
- Klein, C. A., Jr., Walker, J., Swanson, R. C., Caraway, C., and Trygg, J. E. (1974). Epidemiological reports of infectious hepatitis associated with the consumption of oysters. *Proc. Natl. Shellf. Sanit. Workshop*, 8th pp. 53-64.
- Ko, R. C. (1976). Experimental infection of mammals with larval *Echinocephalus sinensis* (Nematoda: Gnathostomatidae) from oysters (*Crassostrea gigas*). *Can. J. Zool.* **54**, 597-609.
- Ko, R. C., Morton, B., and Wong, P. S. (1974). *Echinocephalus* sp. Molin, 1858 (Spiruroidea: Gnathostomatidae), an unusual nematode from the oyster, *Crassostrea gigas* Thunberg, 1793. *Proc. Int. Congr. Parasitol.*, 3rd pp. 1731-1732.
- Ko, R. C., Morton, B., and Wong, P. S. (1975). Prevalence and histopathology of *Echinocephalus sinensis* (Nematoda: Gnathostomatidae) in natural and experimental hosts. *Can. J. Zool.* **53**, 550-559.
- Korringa, P. (1968). Biological consequences of marine pollution with special reference to the North Sea fisheries. *Helgol. Wiss. Meeresunters.* **17**, 126-140.
- Korringa, P., and Roskam, R. T. (1961). An unusual case of mussel poisoning. *Int. Counc. Explor. Sea, Shellfish Comm., Doc. C.M.1961/41*, 2 pp.
- Kourano, M., and Vasquez, M. A. (1975). The first reported case from Panama of acute gastroenteritis caused by *Vibrio parahaemolyticus*. *Am. J. Trop. Med. Hyg.* **24**, 638.
- Krantz, G. E., Colwell, R. R., and Lovelace, E. (1969). *Vibrio parahaemolyticus* from the blue crab *Callinectes sapidus* in Chesapeake Bay. *Science* **164**, 1286-1287.
- Lewis, D. H. (1975). Retention of *Salmonella typhimurium* by certain species of fish and shrimp. *J. Am. Vet. Med. Assoc.* **167**, 551-552.
- Liu, O. C., Seraichekas, H. R., and Murphy, B. L. (1966). Viral pollution and self-cleansing mechanism of hard clams. In "Transmission of Viruses by the Water Route" G. Berg, ed.), pp. 419-437. Wiley (Interscience), New York.
- LoCicero, V. R. (Ed.) (1975). "Proceedings of the First International Conference on Toxic Dinoflagellate Blooms," 541 pp. Mass. Sci. Tech. Found., Wakefield, Massachusetts.
- Lumsden, L. L., Hasseltine, H. E., Leake, J. P., Weldee, M. V. (1925). A typhoid fever epidemic caused by oyster-borne infection (1924-1925). *Public Health Rep. Suppl.* **50**, 1-102.

- Luthy, J. (1979). Epidemic paralytic shellfish poisoning in western Europe, 1976. In "Developments in Marine Biology: Vol. 1. Toxic Dinoflagellate Blooms" (D. L. Taylor and H. H. Seliger, eds.), pp. 15–22. Elsevier/North-Holland, New York.
- Markovic, A. (1939). Der erste Fall von Philophthalmose beim Menschen. *Arch. Ophthalmol.* **140**, 515–526.
- Mason, J. O., and McLean, W. R. (1962). Infectious hepatitis traced to the consumption of raw oysters. An epidemiological study. *Am. J. Hyg.* **75**, 90.
- McFarren, E. F., Schaffer, M. L., Campbell, J. E., Lewis, K. H., Jensen, E. T., and Schantz, E. J. (1961). Public health significance of paralytic shellfish poison. *Adv. Food Res.* **10**, 135–179.
- Mee, L. D., Espinosa, M., and Diaz, G. (1986). Paralytic shellfish poisoning with a *Gymnodinium catenatum* red tide on the Pacific coast of Mexico. *Mar. Environ. Res.* **19**, 77–92.
- Metcalf, T. F., and Stiles, W. C. (1965). The accumulation of enteric viruses by the oyster, *Crassostrea virginica*. *J. Infect. Dis.* **115**, 68–76.
- Metcalf, T. F., and Stiles, W. C. (1966). Survival of enteric viruses in estuary waters and shellfish. In "Transmission of Viruses by the Water Route" (G. Berg, ed.), pp. 439–447. Wiley, (Interscience), New York.
- Miyazaki, I. (1960). On the genus *Gnathostoma* and human gnathostomiasis with special reference to Japan. *Exp. Parasitol.* **9**, 338–370.
- Morse, D. L., Guzewich, J., Hanrahan, J. P., Stricof, R., Shayegani, H., Deibel, R., Grabau, J. C., Nowak, N. A., Herrmann, J. E., Cukor, G., and Blacklow, N. R. (1986). Wide-spread outbreaks of clam- and oyster-associated gastroenteritis—role of Norwalk virus. *N. Engl. J. Med.* **314**, 678–681.
- Murphy, A. M., Grohmann, G. S., Christopher, P. J., Lopez, W. A., Davey, G. R., and Millsom, R. H. (1979). An Australia-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. *Med. J. Aust.* **2**, 329–333.
- National Oceanic and Atmospheric Administration (1976). "Report to Congress on Ocean Dumping Research," 250 pp. Washington, D.C.
- Norris, D. E., and Overstreet, R. M. (1976). The public health implications of larval *Thynnascaris* nematodes from shellfish. *J. Milk Food Technol.* **39**, 47–54.
- Oliver, J. D. (1981). The pathogenicity and ecology of *Vibrio vulnificus*: A particularly virulent microorganism. *Mar. Technol. Soc. J.* **15**, 45–52.
- Oshima, Y., and Yasumoto, T. (1979). Analysis of toxins in cultured *Gonyaulax excavata* cells originating in the Ofurato Bay, Japan. In "Developments in Marine Biology: Vol. 1. Toxic Dinoflagellate Blooms" (D. L. Taylor and H. H. Seliger, eds.), pp. 377–380. Elsevier/North-Holland, New York.
- Overstreet, R. M. (1987). Solving parasite-related problems in cultured crustaceans. *Int. J. Parasitol.* **17**, 309–318.
- Pappalardo, R., and Boemare, N. (1982). An intracellular *Streptococcus*, causative agent of a slowly developing disease in the Mediterranean crab, *Carcinus mediterraneus*. *Aquaculture* **28**, 283–292.
- Peffers, A. S. R., Bailey, J., Barrow, G. I., and Hobbs, B. C. (1973). *Vibrio parahaemolyticus*, gastroenteritis and international air travel. *Lancet* **1**, 143.
- Penner, L. R. (1942). Studies on dermatitis-producing schistosomes in eastern Massachusetts, with emphasis on the status of *Schistosomatium pathlocopticum* Tanabe, 1923. *J. Parasitol.* **28**, 103–116.
- Penner, L. R. (1950). *Cercaria littorinalinae* sp. nov., a dermatitis-producing schistosome larva from the marine snail, *Littorina planaxis* Philippi. *J. Parasitol.* **36**, 466–472.
- Penner, L. R., and Fried, B. (1961). Studies on ocular trematodiasis. I. Marine acquired philophthalmiasis. *J. Parasitol.* **47**, (Suppl 31), (abstract).

- Penner, L. R., and Fried, B. (1963). *Philophthalmus hegneri* sp. n., an ocular trematode from birds. *J. Parasitol.* **49**, 974-977.
- Petter, A. J. (1969a). Enquête sur les nématodes des sardines pêchées dans la région nantaise. Rapport possible avec les granulomes eosinophiles observés chez l'homme dans la région. *Ann. Parasitol.* **44**, 25-35.
- Petter, A. J. (1969b). Enquête sur les nématodes des poissons de la région nantaise. Identification des larves d'ascarides parasitant les sardines (en rapport avec les granulomes eosinophiles observés chez l'homme dans la région). *Ann. Parasitol.* **44**, 559-579.
- Pirquet, K. T. (1988). Poisonous secrets. Shellfish testing in Canada. *Can. Aquacult.* **4**(2), 41-43, 46-47, and 53.
- Porres, J. M., and Fuchs, L. A. (1975). Isolation of *Vibrio parahaemolyticus* from a knee wound. *Clin. Orthop.* **106**, 245-252.
- Ray, S. M., and Wilson, W. B. (1957). The effects of unialgal and bacteria-free cultures of *Gymnodinium brevis* on fish and notes on related studies with bacteria. *U.S. Fish Wildl. Serv., Spec. Sci. Rep.* **211**, 50 pp.
- Reyes-Vasquez, G., Ferraz-Reyes, E., and Vazquez, E. (1979). Toxic dinoflagellate blooms in northeastern Venezuela during 1977. In "Developments in Marine Biology: Vol. 1. Toxic Dinoflagellate Blooms" (D. L. Taylor and H. H. Seliger, eds.), pp. 191-194. Elsevier/North-Holland, New York.
- Richards, G. (1985). Outbreaks of shellfish-associated enteric virus illness in the United States: Requisite for development of viral guidelines. *J. Food Prot.* **48**, 815-823.
- Roos, B. (1956). Hepatitis epidemic transmitted by oysters. *Sven. Laekartidn.* **53**, 989-1003.
- Ruddy, S. J., Johnson, R. F., Mosley, J. W., Atwater, J. B., Rossetti, M. A., and Hart, J. C. (1969). An epidemic of clam-associated hepatitis. *JAMA, J. Am. Med. Assoc.* **208**, 649-655.
- Sakazaki, R., Iwanami, S., and Fukumi, H. (1963). Studies on the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*. I. Morphological, cultural, and biochemical properties and its taxonomical position. *Jpn. J. Med. Sci. Biol.* **16**, 161-188.
- Sato, T., Fukuyama, T., Yamada, M., and Takayagani, J. (1959). Minamata disease. Hg content in poisoned cats, fish and silt from Minamata Bay. *Koshu Eiseiin Kenkyu Hokoku* **8**, 183.
- Shewan, J. M., and Liston, J. (1954). A review of food poisoning caused by fish and fishery products. *Appl. Bacteriol.* **17**, 522.
- Shimizu, Y. (1983). Unexpected developments in red tide research. *Maritimes* **27**(1), 4-6.
- Shimizu, Y., and Yoshioka, M. (1981). Transformation of paralytic shellfish toxins as demonstrated in scallop homogenates. *Science* **212**, 547-549.
- Sindermann, C. J. (1960). Ecological studies of marine dermatitis-producing schistosome larvae in northern New England. *Ecology* **41**, 678-684.
- Sindermann, C. J., and Gibbs, R. F. (1953). A dermatitis-producing schistosome which causes "clam diggers itch" along the central Maine coast. *Maine Dep. Sea Shore Fish., Res. Bull.* **12**, 20 pp.
- Sizemore, R. K. (1986). Involvement of *Vibrio* spp. in soft-crab mortalities. In "Proceedings of the National Symposium on the Soft-Shell Blue Crab Fishery" (H. M. Perry, and R. F. Malone, eds.), pp. 75-78. Gulf Coast Research Lab., Biloxi, Mississippi.
- Smith, R. M. (1971). *Vibrio parahaemolyticus*. *Clin. Med.* **78**, 22-25.
- Smith, W. E., and Smith, A. M. (1975). "Minamata," 220 pp. Holt, Rinehart & Winston, New York.
- Sommer, H., and Meyer, K. F. (1937). Paralytic shellfish poisoning. *Arch. Pathol.* **24**, 560-598.
- Stunkard, H. W., and Hinchliffe, M. G. (1952). The morphology and life-history of *Microbilharzia variglandis* (Miller and Northrup, 1926) Stunkard and Hinchliffe, 1951, avian

- blood-flukes whose larvae cause "swimmer's itch" of ocean beaches. *J. Parasitol.* **38**, 248–265.
- Sullivan, J. J., Iwaoka, W. T., and Liston, J. (1983). Enzymatic transformation of PSP toxins in the littleneck clam (*Protothaca staminea*). *Biochem. Biophys. Res. Commun.* **114**, 465–472.
- Takayama, S., Shirakawa, M., and Hirano, H. (1959). A histopathological investigation of the stomach tumours of a cat experimentally infected with *Gnathostoma spinigerum*. *Jpn. J. Parasitol.* **8**, 829–834.
- Takikawa, I. (1958). Studies on pathogenic halophilic bacteria. *Yokohama Med. Bull.* **9**, 313–322.
- Taylor, D. L., and Seliger, H. H. (Eds.) (1979). "Developments in Marine Biology: Vol. 1. Toxic Dinoflagellate Blooms." Elsevier/North-Holland, New York.
- Twedt, R. M., Spaulding, P. L., and Hall, H. E. (1969). Morphological, cultural, biochemical, and serological comparison of Japanese strains of *Vibrio parahaemolyticus* with related cultures isolated in the United States. *J. Bacteriol.* **98**, 511–518.
- Vanderzant, C., Nickelson, R., and Parker, J. C. (1970). Isolation of *Vibrio parahaemolyticus* from Gulf Coast shrimp. *J. Milk Food Technol.* **33**, 161–162.
- Varga, S., and Hirtle, W. A. (1975). Incidence of *Vibrio parahaemolyticus* in fish, shellfish, mud, water, and fish products in the Canadian Maritime region. *J. Fish. Res. Board Canada* **32**, 541 (*Microbiol. Abstr.* **10**, B11810).
- Vasconcelos, G. J., Stang, W. J., and Laidlaw, R. H. (1975). Isolation of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* from estuarine areas of southeastern Alaska. *Appl. Microbiol.* **29**, 557–559.
- Vermeil, C., Petter, A., Morin, O., LeBodic, M. F., Daniel, C., Guegan, J., and Kerneis, J. P. (1975). Les granulomes eosinophiles signalés en Bretagne representent-ils une forme d'anisakiase? Les larves de *Thynnascaris aduncum* ne permettent pas d'obtenir expérimentalement ces granulomes. *Bull. Soc. Pathol. Exot.* **68**(1), 79–83.
- Vogel, H. (1933). *Himasthla muehlensi* n. sp. ein neuer menschlicher Trematode de Familie Echinostomidae. *Zentralbl. Bakteriol., Parasitenk., Abt. I, Orig.* **127**, 385–391.
- Waldichuk, M. (1988). Mussel toxin leads to shellfish ban. *Mar. Pollut. Bull.* **19**(3), 95–96.
- Wallace, G. D., and Rosen, L. (1966). Studies on eosinophilic meningitis. 2. Experimental infection of shrimp and crabs with *Angiostrongylus cantonensis*. *Am. J. Epidemiol.* **84**, 120–131.
- Wallace, G. D., and Rosen, L. (1967). Studies on eosinophilic meningitis. 4. Experimental infection of fresh-water and marine fish with *Angiostrongylus cantonensis*. *Am. J. Epidemiol.* **85**, 395–402.
- Williams, H. H., and Jones, A. (1976). Marine helminths and human health. *Misc. Publ. — Commonw. Inst. Helminthol.* **3**, 47 pp.
- Yasumoto, T., Oshima, Y., and Yamaguchi, M. (1978). Occurrence of a new type of shellfish poisoning in the Tohoku district. *Nippon Suisan Gakkaishi* **44**, 1249–1255.
- Yasumoto, T., Oshima, Y., Sugawara, W., Fukuyo, Y., Oguri, H., Igarashi, T., and Fujita, N. (1980). Identification of *Dinophysis fortii* as the causative organism of diarrhetic shellfish poisoning. *Nippon Suisan Gakkaishi* **46**, 1405–1411.
- Yokogawa, M. (1964). *Paragonimus* and paragonimiasis. *Prog. Med. Parasitol. Jpn.* **1**, 63–156.
- Young, P. C., and Lowe, D. (1969). Larval nematodes from fish of the subfamily Anisakinae and gastrointestinal lesions in mammals. *J. Comp. Pathol.* **79**, 301–313.
- Zen-Yoji, H., Sakai, S., Terama, T., Kudo, Y., and Hitogoto, H. (1963). Studies of the enteropathogenic halophilic bacteria. I. On the epidemiology of the food poisoning due to halophilic bacteria in Tokyo District during 1961 and bacteriological examination. *J. Jpn. Infect. Dis. Assoc.* **37**, 195–204 (in Japanese; English summary.)

17

Conclusions

A consistent effort has been made throughout this volume to draw conclusions at the end of each chapter—in accord with the objective, stated in Chapter 1, of making each chapter reasonably autonomous, while still contributing to some elusive integrated whole. Despite this approach, though, it seems that space should be provided somewhere for a few overriding generalizations. Those that seem of sufficient stature for such favored treatment include (1) the nature of disease resistance, (2) characteristics of epizootics, (3) disease control, (4) diagnostic methods, and (5) risks of disease from transfers and introductions.

I. DISEASE RESISTANCE IN SHELLFISH

The development of resistance in molluscan shellfish populations as a consequence of massive disease-caused mortalities is indicated by a gradual decline in mortalities, despite a continued high prevalence of infections. This principle has been demonstrated and described in outbreaks of the protozoans *Haplosporidium nelsoni* and *Perkinsus marinus* in American oysters (Ford and Haskin, 1982; Andrews, 1984) and the parasitic copepod *Mytilicola intestinalis* in European mussels (Dethlefsen, 1972). Resistant individuals still become infected, but the severity of infections is less, as are resultant mortalities.

The dim outlines of at least some of the predominant features of the internal defense systems of shellfish are beginning to emerge. Phagocytosis of invading microorganisms by fixed and motile cells is an unquestioned cornerstone. Enhancement of phagocytic activity has been demonstrated. In Mollusca pronounced hemocytosis is an indicator of infections, as are the phenomena of encapsulation of larger parasites and nacreization of mantle irritants.

Humoral defenses seem to be dominated by lytic properties of he-

mocytes and hemolymph, conferred by lysosomal enzymes. Augmented levels of certain of these enzymes have been reported following exposure to foreign proteins.

The later stages of isolation and elimination of pathogens are accomplished in molluscs by the formation of focal tissue abscesses and shell pustules; rupture of abscesses or diapedesis through the mantle epithelium is often accompanied by the formation of nacreized shell pustules (Farley, 1968). The counterpart of these activities which result in eliminating pathogens in crustaceans is seen in the formation of "black gills," characterized by aggregation of phagocytes, melanin deposition, and blockage of the terminal portions of gill filaments—with their subsequent loss at ecdysis.

II. CHARACTERISTICS OF EPIZOOTICS IN SHELLFISH

Epizootic waves in molluscs do not follow the typical vertebrate pattern (an outbreak with high incidence and mass mortalities, followed by a rapid decline to low enzootic levels). Instead, the descending limb of the curve in molluscs is often very gradual and long term, and is punctuated by sporadic resurgences of high prevalences and renewed mortalities (but with peaks usually not reaching the height of the original outbreak). Increased resistance of survivors has been demonstrated, but only after the exposure of several generations to a virulent pathogen.

An examination of the nature of internal resistance factors in invertebrates, as compared with vertebrates, helps to explain the differences seen in epizootic phenomena. In invertebrates responses to foreign proteins are nonspecific and seem to depend principally on preexisting factors in the hemolymph, as well as on phagocytosis. In vertebrates responses are rapid and directed to the specific invading protein, so surviving individuals may be immune to further challenges. In invertebrates individual variability in natural and preexisting factors of resistance may determine survival; in vertebrates individual variability in acquired resistance determines survival or death in the presence of a virulent pathogen.

After examining the available literature, it seems expedient, if not wise, to suggest that epizootics develop in marine shellfish populations as consequences of two reasonably independent phenomena: (1) introduction of a pathogen with which the population has not had previous evolutionary experience, and to which the population is susceptible and (2) massive increases in shellfish populations in localized areas, with attendant increases in stress and facilitation of transmission of pathogens.

III. DISEASE CONTROL IN SHELLFISH POPULATIONS

The original, persistent, and largely erroneous perception about disease in marine populations is that little can be done about it. This pessimistic attitude is definitely unwarranted for species that live inshore, particularly the sedentary invertebrates, for whom practical measures of disease control are possible and have already been applied in some situations. Methods for disease control in invertebrates include the four points listed below.

1. *The transfer of susceptible animals into epizootic areas, or the movement of individuals from such areas, should be prevented.* Because each disease is discrete in terms of transmission and infectivity, risks from transfers will vary as well. When intermediate or alternate hosts play a significant role in maintaining the disease in a given geographical area, transfer of infected individuals to other areas where these hosts are absent may be a reasonable management procedure. Diseases that have been demonstrated experimentally to be transmitted directly, however, such as *Perkinsus marinus* infections of oysters, may be maintained at epizootic levels by repeated introductions of susceptible animals.

2. *Disease-resistant stocks should be developed by selective breeding of survivors.* Epizootics of several oyster diseases have apparently resulted in increased resistance among survivors. During the outbreak of Malpeque Bay disease in oysters of Prince Edward Island (Canada), resistance developed to an unidentified pathogen, and stocks returned to adequate levels of abundance after several decades. That the pathogen is still present is indicated by deaths of susceptible oysters from other geographical areas introduced into Prince Edward Island waters. Resistant stocks were drawn upon to repopulate other oyster-growing areas of the Gulf of St. Lawrence that had been subsequently decimated by the same disease.

Evidence is accumulating that increased resistance to the haplosporidan pathogen, *Haplosporidium nelsoni*, is developing among oysters that have survived the disease in the Middle Atlantic states. The disease has been at epizootic levels in some Delaware Bay and Chesapeake Bay populations for three decades. Resistant strains can be developed with presently available hatchery techniques. Aggregation of survivors on protected natural beds, to which adequate cultch has been added, could also do much to improve reproduction, spatfall, and a return to full production.

3. *Basic information about the life history and ecology of the disease agent must be accumulated,* to define vulnerable stages or restrictive environmental requirements. As an example, several oyster pathogens, such as *H. nelsoni*, are limited to salinities in excess of 15 ‰. Planting during

epizootics can be restricted to low-salinity areas, and temporary transfer of infected stocks to low salinities may retard or eliminate infections.

Mechanical and chemical treatments can also reduce disease prevalence. The effects of gaffkemia on impounded lobster populations have been reduced by treating bottom muds of pounds with calcium hypochlorite. Damage from *P. marinus* disease in oysters has been lessened by planting oysters thinly on the beds, by harvesting within 2 years, and by planting and harvesting at prescribed seasons, to take advantage of the decrease in pathogen activity during the colder months.

The effects of two serious diseases of molluscs in The Netherlands were reduced by knowledge of environmental requirements of the pathogens. Shell disease of oysters, *Ostrea edulis*, was found to be caused by a fungus, *Ostracoblabe implexa*, that perforated the shell and thrived on old shells. An outbreak of shell disease in 1930 in The Netherlands was attributed to the practice of spreading enormous quantities of cockleshells on the beds (Korringa, 1952). The disease declined when the spat collectors were placed in areas free of the disease, when old shells were cleared from beds, and when infected young oysters were dipped in mercuric disinfectant (a procedure that would probably not be recommended now). A second disease, caused by the parasitic copepod *Mytilicola intestinalis* in mussels, was limited in its spread through cultivated stocks by an extensive program outlined by Korringa (1959). Included were the extensive dredging of adjacent natural beds, the transfer of lightly infected stocks, and the destruction of heavily infected beds.

Biological control of other hosts in the life cycle of parasites or of the parasite itself is also a possible approach. Greater attention will undoubtedly be paid in the future to the possibilities of biological control of shellfish diseases—both in wild populations and in aquaculture. Some interesting insights are already appearing:

1. A microsporean hyperparasite, *Nosema ormiersi*, has been reported in the European oyster pathogen *Marteilia refringens* (Comps *et al.*, 1979). The hyperparasite caused necrotic changes in sporogenic stages of *Marteilia*, and reduced the number of spores.
2. An ascetosporan, *Urosporidium spisuili*, hyperparasitizes the larval nematode *Sulcascares sulcata* in surf clams on the Atlantic coast of the United States. An epizootic of the protozoan hyperparasite occurred in the mid-1970s, and a depressing influence on the nematode population was postulated (Perkins *et al.*, 1975).

It seems entirely logical to expect that introduced pathogens may

encounter new biological agents for their destruction in the recipient environment—or that introduced pathogens may transport their own hyperparasites, which may, in turn, become more pathogenic in a new environment.

4. *Production could be maintained in artificial environments, where disease can be controlled.* Some progress has been made in this direction with the development of hatchery methods of producing seed oysters and clams (Loosanoff and Davis, 1963). Bacterial and fungal epizootics in larval culture tanks can be prevented, or their effects reduced, by ultraviolet treatment of filtered seawater, antibiotic treatment of seawater in static or recirculated cultures, the maintenance of general cleanliness of all utensils used in handling larvae, ultraviolet treatment of phytoplankton food derived from impure mass cultures, and the use of bacteria-free phytoplankton cultures or sterilized artificial diets.

Shellfish production in artificial ponds provides distinct opportunities for disease and predator control, beginning with disease-free or disease-resistant brood stock and progressing to filtration and ultraviolet treatment of recirculated water; important also are the careful control of contaminants in mass phytoplankton cultures and the elimination of shellfish associates that act as alternate or intermediate hosts of disease agents.

IV. DIAGNOSTIC METHODS

Generalized disease signs exist in shellfish, just as they do in fish and higher vertebrates. In molluscs mantle recession, pale digestive gland, thin, watery meats, and yellow–green pustules on the mantle are often gross signs of disease. In crustaceans, lethargic and/or disoriented behavior, the cessation of molting, and the proliferation of epibionts often indicate the presence of disease. Beyond these nonspecific indicators, the diagnostic method of choice at present is histopathology. Shellfish pathogens may be confined initially to specific tissues, but often they affect other tissues as the infection progresses. Histopathology can be augmented by culture techniques, but shellfish cell cultures are not available for viral or other studies, and immunological approaches suffer from a lack of specificity of responses.

V. RISKS OF DISEASE FROM TRANSFERS AND INTRODUCTIONS

Transfers and introductions of marine species have occurred and are occurring on a worldwide basis, largely in response to the perceived needs of expanding aquaculture industries. Shrimp and oysters are at production levels in aquaculture, and large-scale movements of repre-

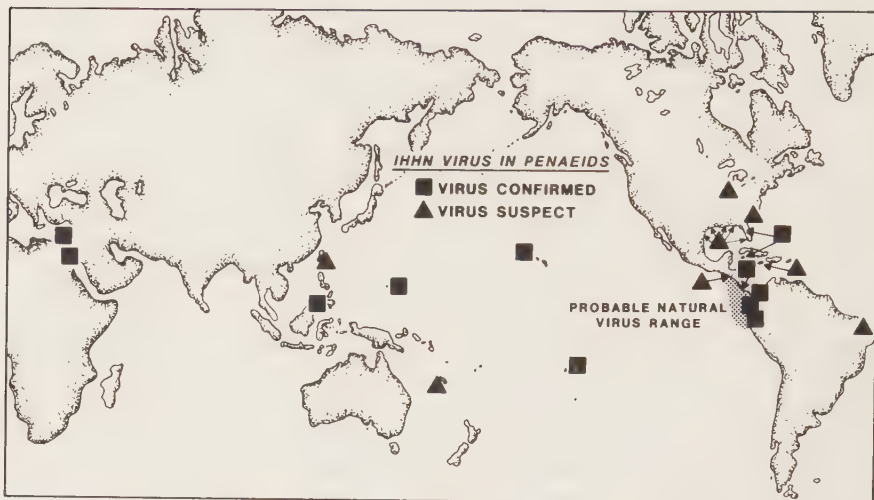


Figure 60. Natural range and global dissemination of infectious hypodermal and hematopoietic necrosis virus of shrimp. Redrawn and modified from Bell and Lightner (1983).

representatives of both groups have occurred, and are occurring, in the perpetual search for the best economic advantage. A major concern is the introduction of diseases not presently enzootic in an area or hydrographic zone. Recent worldwide transfer of a viral pathogen of penaeid shrimp is an excellent example. In the past 10 years, four viral pathogens of shrimp have been recognized: *Baculovirus penaei*, monodon baculovirus, baculoviral midgut gland necrosis, and infectious hypodermal and hematopoietic necrosis virus (IHHNV). Of these, IHHNV is the most recently described and the one that has been disseminated worldwide. IHHNV poses a serious threat to shrimp aquaculture. Found in cultured *Penaeus stylirostris* and *Penaeus vannamei* and considered enzootic on the west coast of Central and South America (Bell and Lightner, 1983, 1984), it has been introduced into aquaculture facilities in Hawaii, Florida, Texas, Tahiti, the Philippines, Guam, and possibly elsewhere (Fig. 60). *Penaeus stylirostris* is especially susceptible, and mortalities from IHHNV infections have been demonstrated. *Penaeus vannamei* harbors latent infections, and mortalities are less common. Experimental infections with IHHNV have been achieved in *Penaeus japonicus* and in *Penaeus monodon*.

IHHNV disease was first seen in 1981 in Hawaiian shrimp culture facilities rearing *P. stylirostris* introduced from Panama. Soon afterward, the role of *P. vannamei* as a carrier of the virus was recognized. The virus

can be observed in juveniles and adults, where it causes its effects, but not in larvae or postlarvae. Of major concern is the possibility of infections of native populations by the introduced pathogen, even though there is no present evidence demonstrating that this has happened. Strict quarantine of imports is extremely important, as is care in the selection of IHNV-free sources of brood stock.

The spread of IHNV will probably become a classic case of the potential for transport of pathogens when marine animals are moved over long distances. Other good examples exist—such as the export of the lobster pathogen, *Aerococcus viridans*, from North America to Europe and the introduction of the Pacific oyster parasitic copepod, *Mytilicola orientalis*, to the coast of France. All of these examples suggest great circumspection when considering the introduction of nonindigenous marine species.

REFERENCES

- Andrews, J. D. (1984). Epizootiology of haplosporidan diseases affecting oysters. *Comp. Pathobiol.* **7**, 243–269.
- Bell, T. A., and Lightner, D. V. (1983). The penaeid shrimp species affected and known geographic distribution of IHNV virus. *Proc. Conf. Warm Water Fish Cult.—Crustaceans*, 1st 12 pp.
- Bell, T. A., and Lightner, D. V. (1984). IHNV virus: Infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. *Aquaculture* **38**, 185–194.
- Comps, M., Pichot, Y., and Deltreil, J.-P. (1979). Mise en évidence d'une microsporidie parasite de *Marteilia refringens* agent de la maladie de la glande digestive de *Ostrea edulis* L. *Rev. Trav. Inst. Pêches Marit.* **43**, 409–412.
- Dethlefsen, V. (1972). Zur Parasitologie der Miesmuschel (*Mytilus edulis* L., 1758). *Ber. Dtsch. Wiss. Komm. Meeresforsch.* **22**, 344–371.
- Farley, C. A. (1968). *Minchinia nelsoni* (Haplosporida) disease syndrome in the American oyster *Crassostrea virginica*. *J. Protozool.* **15**, 585–599.
- Ford, S. E., and Haskin, H. H. (1982). History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957–1980. *J. Invertebr. Pathol.* **40**, 118–141.
- Korringa, P. (1952). Recent advances in oyster biology. *Q. Rev. Biol.* **27**, 266–308 and 339–365.
- Korringa, P. (1959). Checking *Mytilicola*'s advance in the Dutch Waddensea. *Meet. Cons. Perm. Int. Explor. Mer*, 45th, *Shellfish Comm. Rep.* **87**, 3 pp.
- Loosanoff, V. L., and Davis, H. C. (1963). Rearing of bivalve mollusks. *Adv. Mar. Biol.* **1**, 1–136.
- Perkins, F. O., Zwerner, D. E., and Dias, R. K. (1975). The hyperparasite, *Urosporidium spisuli* sp. n. (Haplosporea), and its effects on the surf clam industry. *J. Parasitol.* **61**, 944–949.

•
•
•
•
•
•
•
•
•

•

•

1

9

3

1

11

Epilogue

Writing these two volumes of *Principal Diseases of Marine Fish and Shellfish* has been a remarkable experience—probably best described as “demanding but intellectually stimulating.” Creation of each chapter provided a source of satisfaction—even at times exhilaration—although the results may be better in some instances than in others. There has been great satisfaction too in assembling and examining the original literature, old and new, in each subject matter area delineated by the chapter headings.

The orientation of the book, toward diseases of importance to economic species of marine fish and shellfish, arbitrarily excluded much descriptive material that fell outside that narrow perimeter. Fortunately, the extensive multi-volume compendium of information on *all* marine species edited by Dr. Otto Kinne (*Diseases of Marine Animals*) is now almost complete, and most of it has been published. Thus, the generalist and the specialist have choices, not only with these books but also with others in the growing specialty areas of marine pathology. Symposium volumes and other edited volumes, as well as extensive reviews, have been appearing with increasing frequency; these can also be sources of excellent authoritative information.

Among the insights gained from writing this book is the sense of continuity in the development of information about diseases and disease agents. Some of the better-known pathogens now have a history of more than a century of research and publications, often marked by the efforts of several generations of investigators down through the decades. Other disease agents—especially the viruses—have much shorter research histories; undoubtedly, many other agents are still in the wings, waiting for their time onstage.

Another insight relates to the obvious dedication of a special group of investigators to long-term examination of a single disease, parasite, or

parasite group. Probably the most outstanding example of this is Weisenberg's persistence in studying lymphocystis disease of fish for more than half a century. Other examples would be Wolf's contributions to fish virus research over 25 years, Kabata's attention to parasitic copepods over a period of more than three decades, and Hargis' examination of the Monogenea over a similar period.

Of course, the literature also abounds with another type of contribution—several years of intensive and often excellent publications and then silence—leaving the reviewer forever uncertain about the reasons. Did the investigator simply move on to other more fruitful areas of research? Did he or she take a better-paying industry or administrative position? Did he or she retire or die suddenly? Answers to these questions are, of course, outside the purview of a scientific reviewer, but they are important in pointing to the existence of a human side of science, and scientists, which rarely invades the technical literature.

Somewhere among these final words a niche should exist to give some final applause to fish and shellfish pathologists, many of whom are still much enmeshed in the purely descriptive side of their specialty (identification of new pathogens, life cycle studies, host-parasite interactions, and pathogen-environment interactions, for example), but most of whom are expected to address practical disease control issues and to accumulate robust statistical data on disease effects. Progress is evident. Disease is clearly an important factor in marine populations, but the research horizons are still close at hand.

And now it is time to readdress the five basic questions asked in the preface to volume 1, questions with answers that have been approached gingerly from different perspectives in all of the chapters of these two volumes:

1. What *are* the important diseases of marine fish and shellfish?
2. What are the *effects* of disease on wild and cultivated populations of fish and shellfish?
3. How do fish and shellfish resist invasion by potential pathogens?
4. Has coastal/estuarine pollution had any influence on fish and shellfish diseases?
5. What are the public health implications of fish and shellfish diseases?

An answer to the first question is relatively easy, once a substantial fragment of the literature has been digested and assimilated. The systematic reviews in the early chapters of both volumes should provide much of the information needed for assessments of worth, accepting the

ultimate subjectivity of any conclusions reached, whether by the author or by the reader.

The second question is tougher to answer. Some good information on effects has been presented, but it is often vaguely unsatisfactory from a quantitative viewpoint. Marine pathologists tend to tiptoe around, or otherwise avoid, numbers and complex population models, although that situation is changing slowly as long-term data bases improve.

The third question has received substantial attention during recent decades, and these chapters are among the longest in both volumes. Understanding of immune responses of fish has progressed remarkably, and insights have been gained about internal defenses of shellfish—although much remains to be elucidated.

The fourth question has no clear answers at present, except for the obvious one that pollution, as a form of environmental stress, clearly impinges on the disease process in marine animals. Specific examples of associations of pollution and disease have been examined, and some statistical relationships have been proposed, but many conclusions must be labeled “inferential.”

The final question leads to interesting and somewhat surprising findings, indicating a very narrow range of overlap of fish and shellfish diseases with human diseases. A few problem areas do exist, particularly with some of the helminths transmissible to humans, but they are remarkably infrequent.

And with this, the trip ends. Information on fish and shellfish diseases is accumulating rapidly—probably faster than these pages can be read. This book is one man’s attempt to stop the world and to capture and distill the present status of knowledge in a narrow specialty area; it even includes some conclusions that seem warranted by the published data—conclusions that, in someone else’s book a decade from now, may be either supported or rejected. The experience of putting it all together has been a professionally rewarding one, providing, as it did, a small window on the expanding universe of fish and shellfish diseases.

Subject Index

A

- Aber disease, in flat oyster, *see* *Marteilia refringens*
- Abnormalities, 235–242
 - common causes, 235–236
 - in crustaceans, 239–241
 - in molluscs, 236–239
 - pollution and, 426–427
- Acanthoparyphium spinulosum*, metacercariae in American oyster, 150
- Achromobacter*, mass mortalities in oysters and, 44
- Adamsia palliata* (coelenterate), toxic effect on hermit crabs, 280
- Aerococcus viridans*
 - experimental infection of crabs, 55–56, 287–288
 - gaffkemia induction in lobsters, 41, 53–56, 60, 268, 281–288
 - antibiotic effects on, 287, 334
 - epizootics, 53, 281, 320, 391–393
 - growth stimulation by lobster serum, 55, 282
 - host–parasite relationship, 55, 268, 284–286
 - in impounded populations, 305
 - vaccination and, 56, 287, 333
- taxonomic changes, 54
- in crustaceans, 277–278, 284, 287
- in molluscs, 254–255
- Agmasoma penaei*, in penaeid gonads, 121, 122
- Ameson michaelis*, in blue crab muscles, 124
- A. nelsoni*, cotton shrimp condition induction, 118, 120–121
- A. pulvis*, in shore crab muscles, 124
- Amino acid ratio, response to stress in shellfish, 332, 417

- Amusium balloti*, infection by nematode *Sulcascaris sulcata*, 161
- Angiostrongylus cantonensis*, larvae in shellfish, human meningitis and, 467–468
- Antibiotics
 - gaffkemia in lobsters and, 287, 334
 - gram-negative bacteria in molluscs and, 312, 313
- Aphanomyces astaci*, freshwater crayfish plague and, 85, 86, 389–391
- A. ovidestruens*, epizootics in copepod, 395
- Argea pugettensis*, parasitizing black-tailed shrimp, 196
- Argopecten* spp., infection by nematode *Sulcascaris sulcata*, 161
- Ascarophis*, larvae in American lobster, 166
- Ascetosporans
 - in clams, 114
 - in crabs, 128–129
 - in oysters, 97–105, 108–110
 - in shipworms, 115
- Atkinsiella dubia*, *see* *Plectospira dubia*
- Austrobilharzia variglandis* (avian schistosome), in marine snail, 155
 - human dermatitis induction, 463–465
 - life cycle, 464
- Avian schistosomes, in marine snails,
 - human dermatitis and, 155, 463–465

B

- Bacterial diseases, *see also* specific bacteria
 - in crustaceans
 - chlamydial and rickettsial infections, 60–62
 - epibionts, 58–59
 - gaffkemia, 53–56, 60

- Bacterial diseases (*cont.*)
- shell disease, 48–53, 305–306, 319–320
 - significance, 59–60
 - vibriosis, 57–58, 59–60, 306–307
 - in cultured shellfish, importance, 324–325
 - human, transmitted by shellfish, 462–463, 471–472
 - in molluscs, 41–48
 - chlamydial and rickettsial infections, 60–62
 - focal necrosis, 44, 45
 - Pseudomonas* infection, 43–44, 46–47
 - vibriosis, 42–48
 - antibiotic effects, 312
- Bactericidal activity
- in lobsters
 - against gram-negative bacteria, 275
 - against nonpathogenic *Pseudomonas perolens*, 284–285
 - in polychaete, induction by toxic chemicals, 433–434
- Baculovirus penaei*, in shrimp, 21, 27, 29, 30, 34, 318
- latent infection, activation by stress, 422
- Black gill syndrome, in crustaceans, 429–431
- Bonamia ostreae*, in flat oyster, 96, 110–111, 317, 370
- chemotherapy, 335
 - economic losses, 449–450
- Bopyrus fougerouxii*, parasitizing prawn, 196
- B. squillarum*, parasitizing shrimp, 196
- Briarosaccus callosus*, rootlets in king crab, 192–193
- Brown spot disease, bacterial, in prawn, 51, 314
- Bucephalus cuculus*, larvae in American oyster, 147–149
- amino acid ratio change induction, 332, 417
 - fatty acids toxic for humans, 465
 - hyperparasitization by protozoans, 149, 150
- B. longicornutus*, larvae in New Zealand oyster, 149
- hyperparasitization by protozoans, 149–150
- B. margaritae*, metacercariae in pearl oysters, 150–151
- B. varicus*, metacercariae in pearl oysters, 150
- Burn spot disease, bacterial, in edible crab, 49
- C
- Cadmium
- gill lesion induction, shrimps, 428, 431
 - in seafood, public health and, 478
- Callinectes sapidus* (blue crab)
- abnormalities in coloration, 240
 - in culture, disease effects, 322
 - microbial diseases
 - bacterial
 - shell disease, 49–50
 - vibriosis, 58, 349, 460
 - shedding operations and, 307
 - fungal, 79, 80–81
 - viral, 21, 28–31, 33
 - parasites
 - crustacean *Loxothylacus texanus*, 191, 192
 - helminths
 - Carcinonemertes carcinophila* on eggs, 166
 - Microphallus nicolli*, 163
 - Myzobdella lugubris*, 167–168
 - protozoans
 - ciliates in gills, 127, 128
 - dinoflagellate in hemolymph, 126
 - microsporeans in muscles, 124
 - paramoebiasis, 124–126, 272, 393, 451
 - phage clearance, 280
- Cancer irroratus* (rock crab)
- bacterial diseases
 - experimentally induced by *Aerococcus viridans*, 55–56, 287–288
 - vibriosis, 58
 - experimentally induced, 281
 - infection by
 - copepod *Choniosphaera cancrrorum*, 197
 - Polymorphus major* (acanthocephalan) larvae, 165
 - pollution effects
 - black gill disease and, 421
 - shell disease and, 419

- C. magister* (Dungeness crab), infection by
Carcinonemertes errans on eggs, 167
 chlamydia, 61
 phycomycete, 80–81
 trematode metacercariae, 164
- C. pagurus*, bacterial burn spot disease, 49
- Carcinobdella kanibir*, in Japanese edible crab, 168
- Carcinoecetes conformis*, in crab intestinal caeca, 128
- Carcinonemertes carcinophila*, on blue crab eggs, 166
- C. errans*, on Dungeness crab eggs, 166, 167
- Carcinus maenas* (green crab)
 microbial diseases
 baculoviruses and, 29, 30
 vibriosis, 58
 phage clearance, 279–280
 protozoan parasites
 ciliates, 126
 dinoflagellates, 126
 microsporeans, 124
Sacculina carcans rootlets, 191
- C. mediterraneus*, infection by
Streptococcus faecalis, public health and, 466
 rickettsias and, 61
 virus, 31
- Cephaloidophora* (gregarine), in crabs, 127–128
- Cephalolobus* spp., in penaeid stomach, 123
- Cerastoderma edule* (European cockle), hemocytic neoplasia, 221–222, 419
- Cercaria* spp., orange sickness-inducing in mussels, 152
- Cherax destructor*, natural hemagglutinins, 277
- Chionoecetes bairdi* (tanner crab), infection by
 ascomycete *Trichomaris invadens*, 76, 79, 83–84, 451–452
 dinoflagellate *Hematodinium perezii*, 126
- C. opilio*, infestation by *Carcinobdella kanibir*, 168
- C. tanneri*, bacterial shell disease, 49
- Chlamydial infections, molluscs and crustaceans, 60–62
- Cholera, shellfish-related outbreaks, 462–463, 471–472
- Choniosphaera* spp. (copepods), parasitizing crabs, 197
- Ciliates
 in crabs, 126–127, 128
 in lobsters, 128
 in shrimp, 123
- Cliona* (sponge), boring oyster shell, 238
- Copper, phagocytosis activation in oysters, 433
- Corynosoma*, larvae in American lobster, 165
- Cotton shrimp, microsporean-induced, 118–119 (table), 120–123
- Crago nigricauda*, parasitization by isopod, 196
- Crassostrea angulata* (Portuguese oyster)
 fungal shell disease, 72, 73, 368–369
 iridovirus-induced gill disease, 11, 16, 19, 34, 368, 369, 370
 Pacific oyster introduction and, 316, 339–340, 347, 399
 rickettsial infection, 61
- C. commercialis*, see *Saccostrea commercialis*
- C. gigas* (Pacific oyster)
 benign tumors, 224–225
 importation from Japan, effect on native oyster diseases
 in France, 316–317, 337, 340, 347, 399
 on US west coast, 337–338
 mass mortalities
 in Japan, 371–374
 summer, on US west coast, 379–380
 microbial diseases
 Achromobacter, 44
 iridovirus in larval velar epithelium, 11, 17–18, 34
 Pseudomonas spp., 43, 44
 rickettsial, 61
 vibriosis, 42, 44, 45
- parasites
 copepod *Mytilicola orientalis*, 187–188, 337, 495
 helminths
 Echinocephalus sinensis, 159, 467
 Gymnophalloides tokiensis, 150
 Polydora, 162
 Tylocephalum larvae, 155–157
 protozoans
 Haplosporidium-like organism, 105
 Labrynthomyxa-like organism, 116

- C. gigas* (Pacific oyster) (cont.)
 Marteilia refringens, 109
 Marteilioides chungmuensis, 117
 pollution-induced shell abnormalities, 426
- C. glomerata*, mortalities in French Polynesia, 382
- C. iridescens*, paralytic shellfish poisoning and, 474
- C. virginica* (American oyster)
 abnormalities, ectoparasitic *Odostomia* and, 237
 benign tumors, 224
 control of mass mortalities, 382–384
 in culture, disease effects, 322–323
 hemocytic neoplasia, malignant, 220
 Malpeque Bay disease, 338, 339, 374–375, 399
 resistance to, 265
 microbial diseases
 herpes-type virus and, 10, 17, 18, 423
 fungal, 75
 vibriosis, 42–43
 parasites
 crab *Pinnotheres ostreum*, 189
 helminths
 Acanthoparyphium spinulosum, 150
 Bucephalus cuculus, 147–150, 332, 417, 465
 Tylocephalum larvae, 155–157
 pollution effects
 carcinogens and, 429
 developmental abnormalities in embryo, 427
 histopathology, crude oil-induced, 419
 pesticides and, 429, 434
 West River disease during cold-air storage, 308
 protozoan infections
 Haplosporidium costale, 103–105, 315, 338–339, 379
 H. nelsoni
 in culture, 315–317, 338–339, 447–448, 453
 epizootics on US east coast, 95, 97–103, 376–378
 resistance, development of, 256–264, 333, 383, 489, 491
 Hexamita nelsoni, 113
 Labyrinthomyxa-like organism, 116
 Perkinsus marinus
 epizootics on US east coast, 96, 105, 315, 338–339, 375–376, 448–449
 mortalities after introduction to Hawaii and, 381
 resistance development, 265–267, 489
 stock manipulation in culture and, 317, 343–344, 346, 347
- Crayfish plague, oomycete-induced, in Europe, 85, 86, 389–391, 399
- Crustaceans
 abnormalities, 239–241
 infectious diseases
 bacterial, 48–62, 281–288, 320
 chemotherapy in culture, 334–335
 fungal, 76–86, 314, 320, 394–395
 helminth-induced, 162–169
 in impounded populations, 304–307
 protozoan-induced, 117–130
 in crabs, 123–129
 in lobsters, 128, 129–130
 in shrimp, 118–119 (table), 120–123
 viral, 20–34, 450, 494–495
 internal defense mechanisms
 in American lobster infected by *Aerococcus viridans*, 281–288
 cellular, 267–274
 humoral, 267–268, 274–281
 mass mortalities, 388–397
 neoplasms
 benign, 226–227
 malignant, 227–228
 parasitic, 183–198
 copepods
 on lobsters and crabs, 197
 in molluscs, 184–188, 198
 crabs, in molluscs, 188–190
 isopods, in crustaceans, 193–196
 rhizocephalans, in crabs, 190–193, 197–198
 pollution effects, 416, 419–422, 426
 black gill syndrome and, 429–431
 Cryptocotyle lingua, larvae in periwinkle, 154, 360
- Cultured shellfish diseases
 control
 biological, hyperparasitism by protozoans, 160–161, 163, 492–493

chemotherapy, 312, 333–335
 diagnostic services, 329–330, 493
 environmental manipulation, 344–346, 491–492
 principal elements, 326–329, 347–350, 491–493
 regulation of transfers and introductions, 335–343
 Code of Practice, revised, 341–343
 stock manipulation, 342–344, 491
 stress management, 330–332
 vaccination, resistance enhancement and, 332–333
 epizootics, case histories
 lobsters, 319–321
 oysters, 315–317
 shrimp, 314, 317–319
 importance, assessment of
 bacterial infection, 324–325
 environmental influences on larval development, scheme, 326
 for groups of species, 321–323
 viral infections, 323–324, 450

D

Delaware Bay disease, epizootics in oysters, *see* *Haplosporidium nelsoni*
 Depuration plant, shellfish microbial decontamination, 472–473
 Dermatitis, human, trematode-induced, 463–465
Dermocystidium marinum (fungus), *see* *Perkinsus marinus* (protozoan)
Diaptomus gracilis, elimination by phycomycete, 395
Dichelopandalus leptocerus, infection by chytrid-like fungi, 82
Didymaria palinuri, in lobster, 84
 Dinoflagellates
 crab infections, 126
 shellfish poisoning induction in humans, 473–477
Distomum (*Gymnophallus*) spp., pearl formation-inducing in mussels, 151
 Dithiocarbamate biocides, black gill syndrome induction in shrimps, 429–431
 Domoic acid, in mussels, 474–475
Donax, infection by
 dinoflagellate, paralytic shellfish poisoning in humans, 474

Postmonorchis donacis larvae, 153
D. trunculus, infection by hyperparasitized trematode, 153
D. vittatus, infection by trematode, 153
 hyperparasitization by *Nosema legeri*, 154
Dosidicus gigas, infection by cestodes, 158

E

Echeneibothrium, larvae in clams, 157
Echinocephalus pseudouncinatus, larvae in California pink abalone, 159
E. sinensis, larvae in Pacific oyster, 159
 public health and, 467
Eledone cirrhosa, fungal infection, 75
 Encapsulation, reaction by molluscs to helminth parasites, 251–252
 Endotoxins, gram-negative bacterial, crustacean responses to, 274–276, 281
Entoniscus japonicus, parasitizing porcellanid crab, 194
Ephelota gemmipara, on Norwegian lobster eggs, 129
Epipenaeon japonicus, parasitizing prawns, 196
Epistylis sp., on shrimp, chemotherapy, 334
Eupagurus prideauxii, resistance to injected coelenterate toxin, 280
Eurytemora hirundoides (copepod), fungal epizootics, 394

F

Focal necrosis, bacterial, in oysters, 44, 45
 Fungal diseases, *see also* *specific fungi*
 in crustaceans, 76–86
 ascomycetes, 76, 82–83
 characteristics, 77–79 (table)
 chemotherapy, 334–335
 chytrid-like fungi, 82, 83
 Fusarium, 76, 83–84
 phycomycetes, 80–82, 314, 320, 395
 trichomycetes, 84–85
 in molluscs, 71–76
 byssus invasion, 73–74
 phycomycetes, 72–75
 protist parasites, 75–76
 shell disease, 72–73, 85
 mortalities, 366–369, 492

- Fusarium (Ramularia) branchialis*, in lobster, 84
- F. solani*, crustacean infection by, 76–78, 83–84, 320
- resistance to chemotherapy, 335
- G**
- Gaffkemia, *Aerococcus viridans*-induced
- in crabs, experimental infection, 55–56, 287–288
- in lobsters, 41, 53–56, 60, 281–288
- antibiotic effects on, 287, 334
- defense mechanisms, absence of in lobsters, 55–56, 281–282, 284
- description, 54–55
- epizootics, 53, 281, 320, 391–393
- host–parasite relationship, 55, 268, 284–286
- impounded populations and, 305
- infection course, 282–283
- vaccination effect, 56, 287, 333
- Gaffkya homari*, see *Aerococcus viridans*
- Gammarus oceanicus*, PCB effect, 429
- Gastrointestinal outbreaks, human
- Norwalk virus and, 471
- trematodes and, 465
- Vibrio parahaemolyticus* and, 459–462, 471, 480
- in American oyster, 220
- in clams, 223
- Gill disease, viral, in oysters, 11, 16, 316
- Glucose, in crustacean hemolymph, stress effect on, 332, 433
- Gnorimosphaeroma oregonensis*, infection by ciliate *Paranophrys*, 127
- Gray crab disease (paramoebiosis), in blue crab, 124–126, 272, 393
- Gregarines
- in crabs, 127–128
- in oysters, 112
- in shrimp, 123
- Gymnodinium catenatum*, paralytic shellfish poisoning induction, 474
- Gymnophalloides tokiensis*, metacercariae in Pacific oysters, 150
- Gymnophallus bursicola*, pearl inducing in mussels, 151
- G. strigatus*, metacercariae in clam, hyper-parasitization by protozoan, 154
- H**
- Haliotis corrugata*, infection by nematode *Echinocephalus pseudouncinatus*, 159
- H. cracherodii*, pollution effects, 417
- H. laevigata*, infection by *Perkinsus* sp., 115
- H. ruber*, infection by *Perkinsus* sp., 115
- H. rufescens* (red abalone)
- fungal infection, 75
- vibriosis, 47–48
- H. sieboldii*, fungal epizootic in storage facility, 74, 308
- Haliphthoros milfordensis* (phycomycete)
- in abalone held in storage facility, 74, 308
- in crustaceans, 81, 320
- chemotherapy in lobsters, 334
- in oyster drill eggs, 74
- H. philippinensis*, in tiger prawn, 81
- Haplosporidium*
- like organism, in oysters, 105
- in shipworms, 115
- H. costale*, in oysters
- distinction from *H. nelsoni*, 104
- epizootics on US east coast, 95, 103–105, 315, 338–339, 379
- H. louisiana*, in mud crab, 128
- H. nelsoni*, in oysters
- control measures in culture, 317
- distinction from *H. costale*, 104
- epizootics on US east coast, 95, 97–103, 376–378, 399
- in culture, 315–316, 338–339
- economic losses, 447–448, 453
- resistance development, 256–264, 333, 383, 489, 491
- salinity and, 98–99, 103, 261–262, 382–383
- temperature and, 99–100, 260–261, 383
- hemocytosis induction in host, 252–253
- life cycle, 101
- localization in tissues, 100–102
- phagocytosis by host hemocytes, 251, 262, 263
- H. tumefaciens*, in California mussels, 114
- Helminths, see also specific helminths
- in crustaceans, 162–169
- acanthocephalans, 165
- annelids, 167–168
- cestodes, 164–165

- nematodes, 165–166
- nemertean, 166–167
- trematodes, 163–164
 - human diseases and, 465
- turbellarians, 165
- in molluscs, 145–152
 - cestodes, 155–158
 - nematodes, 158–161
 - human diseases and, 467–468
 - trematodes, 146–155
 - human disease and, 463–466
- Hemagglutinins
 - in crustaceans, 277
 - in molluscs, 253–254
- Hematodinium perezii* (dinoflagellate), in crabs, 126
- Hematopoietic neoplasm, *see* Hemocytic neoplasia
- Hemiarthrus abdominalis*, parasitizing shrimp, 196
- Hemigrapsus oregonensis* (shore crab), parasitization by isopod, 194
- Hemocytic neoplasia, molluscan
 - in cockles, 221–222
 - description, 211–216
 - epizootics in bivalves, 222–223
 - in mussels, 220–221
 - in oysters, 219–220
 - in soft-shell clams, 16–17, 216–219, 222, 363–364, 399
 - economic losses, 451
 - pollution and, 423–424
 - terminology, occurrence, 211–216
- Hemocytic sarcoma, *see* Hemocytic neoplasia
- Hemocytosis, in response to infection
 - by crustaceans, 271
 - by molluscs, 252–253
- Herpes-type virus, in oyster larvae, 10, 17, 18
 - proliferation at higher temperature, 422–423
- Hexamita nelsoni* (flagellate), in oysters, 112–113
 - phagocytosis by host hemocytes, 251
- Himasthla muelhensi*, in clams, human infection, 465
- H. quissetensis*, in clams, human infection, 465
- Histiobdella homari*, on American and Norwegian lobsters, 168
- Homarus americanus* (American lobster)
 - abnormalities
 - bicoloration, 239, 240
 - skeletal, 241
 - bacterial diseases
 - gaffkemia, *Aerococcus viridans*-induced
 - in culture, 320–321, 333
 - description, 41, 53–56, 60
 - in impounded populations, 305
 - internal responses, 281–288
 - internal defenses to most bacteria
 - other than *Aerococcus*, 284
 - shell disease, 51–53
 - in culture, 319–320
 - in impounded populations, 305–306
 - vibriosis, 58, 306, 460
 - chytrid-like fungal infection, 82–83
 - in culture
 - diseases, role of, 322
 - epizootics, 319–321
 - rearing techniques, 313
 - humoral responses to infection, 275, 278–279
 - parasites
 - copepod *Unicaleuthes*, 197
 - helminths
 - Ascarophis* larvae, 166
 - Corynosoma* larvae, 165
 - Pseudocarcinonemertes homari*, 167
 - Stichocotyle nephropis*, 164
 - protozoans
 - Paramoeba perniciosa*, 126
 - Paranophrys* spp., 127
 - Porospora* spp., 129
 - pollution effects
 - black gill disease and, 421
 - gill browning, petroleum contamination and, 428–429
 - phosphorus-induced pathology, 428
 - shell disease and, 419
 - tumor-like growth, 226–227
- H. gammarus* (European lobster)
 - abnormalities, bicoloration, 239–240
 - infectious diseases
 - bacterial
 - gaffkemia, 53, 54, 305, 320, 391
 - shell erosion, 52
 - copepod *Nicothoë astaci* and, 197
 - fungal, 84, 320–321
 - Hyaloklossia pelseneeri*, in clams, 114

- Hydractinia echinata*, scallop shell deformity induction, 236–237
Hyphochytrium peniliae, epizootics in copepod, 395
Hysterothylacium (nematode), larvae in *Pandalus borealis*, 166
 public health and, 468

I

- IHHNV, *see* Infectious hypodermal and hematopoietic necrosis virus of shrimp
Illex illecebrosus, infection by nematodes, 161
 protists, 75
 Impounded shellfish infectious diseases
 consequences of injuries during capture, 305–306
 control methods, 308–309
 facultative microbial pathogen-induced, 306–307
 preexisting, 304–305
 reasons for holding operations, 303–304
 Infectious hypodermal and hematopoietic necrosis virus (IHHNV), in penaeid shrimp, 450, 494–495
 Internal defense mechanisms in crustaceans
 American lobster response to *Aerococcus viridans*, 281–288
 cellular responses, 267–274
 humoral responses, 267–268, 274–281
 in invertebrates, 247–249, 288–289
 in molluscs
 American oyster response to protozoan infection, 256–267
 cellular responses, 249–253
 humoral responses, 249–250, 253–256
 Iridoviruses
 in octopus, 10, 18
 in oysters, gill disease induction, 11, 16–17, 316, 339–340, 369–370
Isonema sp., in clams, 115
 Isopods, parasitic
 bopyrids, in shrimps and prawns, 193, 195–196
 cryptoniscids, in rhizocephalan-parasitized hermit crabs, 193
 entoniscids, in crabs, 193–195

K

- Krebspest, *see* Crayfish plague
Kronborgia caridicola, larvae in North Atlantic shrimp, 165

L

- Labyrinthomyxa*
 -like organisms, in oysters, 116–117
L. marina, *see* *Perkinsus marinus*
Labyrinthuloides haliotidis, in juvenile abalones, 75
Lagenidium (phycomycete), in crustaceans, chemotherapy, 334
L. callinecetes, in crustacean eggs and larvae, 77–79 (table), 80–81, 320
L. marina, in pea crab, 81
Lagenophrys (ciliate), on blue crab, 127, 128
L. lunatus, on shrimp, 123
Leander serrifer (shrimp), parasitization by isopod, 196
 Leeches, parasitizing crustaceans, 167–168
Leucothrix mucor, on crustaceans, 58–59
Liriopsis pygmaea, hyperparasitization of *Peltoaster paguri* in hermit crabs, 193
Lithodes spp., infection by rhizocephalans, 193
Littorina (periwinkle), infection by larval trematodes, 154, 155
L. littorea, infection by larval *Cryptocotyle lingua*, 154, 360
L. saxatilis, pollution effects on embryos, 427
Loligo pealei, vibriosis, 48
Loxothylacus texanus, parasitic in blue crab, 191, 192
 Lysosomal enzymes, in molluscan hemolymph, 254–255

M

- Macoma balthica* (duck clam), gill carcinoma, epizootics, 222, 223, 425
Macrobrachium rosenbergii (Malaysian prawn), diseases in culture, 322
Macropipus depurator, infection by virus, 20, 24, 30
Mactra veneriformis, infection by copepod, 188

- Malachite green, microbial infection inhibition in crustaceans, 334
- Malpeque Bay disease, in oysters, pathogen unknown
- epizootics, 338, 339, 374–375, 399
 - resistance to, 265
- Marteilia maurini*, in mussels, 114
- M. refringens*, in flat oyster
- control measures in culture, 317
 - digestive gland disease induction, 95, 108–110
 - epizootics, 316, 369–370
 - economic losses in culture, 449–450
 - hyperparasitization by *Nosema ormierei*, 492
- M. sydneyi*, in Sydney rock oyster, 95, 111, 381
- Marteilioides chungmuensis*, in Pacific oyster oocytes, 117
- Mass mortalities
- in crustaceans, 388–397
 - common features, 396–397
 - copepod fungal diseases, 393–394
 - in crayfish plague, in Europe, 85, 86, 389–391, 399
 - gaffkemia in lobsters, 391–393
 - gray crab disease in blue crab, 393
 - shrimp diseases in culture, 395
 - small noneconomic organisms, diseases, 394–395
 - in molluscs, 361–364
 - oyster epizootics, 364–388
 - in Asia, 371–374
 - in Australia, 380–381
 - definition, 365
 - in Europe, 366–371
 - generalizations, 384–388
 - in North America, 374–380
 - occurrence during past century, 367 (table)
 - in Pacific islands, 381–382
- Melanization, response to infection in crustaceans, 273
- Meningitis, eosinophilic, nematode-induced, marine animals and, 467–468
- Mercenaria mercenaria* (hard-shell clam)
- abnormalities, 238–239
 - gonadal neoplasms, malignant, 223, 425
 - infection by
 - chlamydia, 61
 - Himasthla quissetensis*, fatty acids toxic for humans, 465
 - Polydora*, amino acid ratio and, 331–332, 417
- mass mortalities, unknown etiology, 363
 - pollution effects
 - disease resistance and, 433
 - petroleum-induced histopathology, 428, 431–432
- Mercury, in seafood, public health and, 477
- Microphallus* spp., metacercariae in crabs, 163–164
- Microsporeans
- in clams, trematode-hyper parasitizing, 153–154
 - in crabs, 124
 - trematode-hyperparasitizing, 163
 - in mussels, 113–114
 - in shrimp, 118–119 (table), 120–123
- Microsporidium* sp., cotton shrimp condition induction in pandalid shrimp, 122
- Minchinia nelsoni*, see *Haplosporidium nelsoni*
- Molluscs
- abnormalities, 236–239
 - infectious diseases
 - chemotherapy in culture, 334–335
 - crustacean parasites
 - copepods, 184–188, 198
 - crabs, 188–190
- helminth parasites, 145–162
 - cestodes, 155–158
 - nematodes, 158–161
 - trematodes, 146–155
- in impounded populations, 308
 - microbial
 - agents as facultative or obligate pathogens, 324–325
 - bacterial, 41–48, 60–62
 - fungal, 71–76, 312–313
 - viral, 10–20, 34, 316
- protozoan parasites, 93–117
 - in clams, 114–115
 - in mussels, 113–114
 - in oysters, 95–113, 315–317
- internal defense mechanisms
 - in American oysters infected by protozoans, 256–267
 - cellular, 249–253
 - humoral, 249–250, 253–256

- Molluscs (*cont.*)
- mass mortalities, 361–364
 - oyster epizootics, 364–388
 - neoplasms in bivalves
 - benign, 223–225
 - malignant, 16–17, 211–226
 - in cockles, 221–222
 - epizootics, 222–223
 - in mussels, 220–221
 - in oysters, 219–220
 - in soft-shell clams, 216–219
 - pollution effects, 415–419, 423–426
 - lesions induced by chemicals, 428–429, 431–432
 - Monilia*, shell disease induction in oysters, 72
 - Mottling disease, fungal, in lobsters, 82, 83
 - MSX disease, *See* *Haplosporidium nelsoni*, in oysters
 - Mutations, pollution effects, molluscs, 426–427
 - Mya arenaria* (soft-shell clam)
 - abnormal siphons, 238
 - infectious diseases
 - helminth-induced
 - Cercaria myae* in gondads and, 152
 - Himasthla* spp. and, 152–153
 - rickettsias and, 61
 - malignant neoplasms
 - gonadal, 223
 - hemocytic
 - B-type retrovirus isolation, 16–17, 217, 424
 - epizootics, 217–219, 222, 363–364, 399, 451
 - histopathology, 216
 - pollution effects
 - growth retardation, 417
 - neoplasms and, 423–424
 - Myliobatis californicus*, infection by *Eche-neiobothrium*, 157
 - Mytilicola intestinalis* (copepod), in Euro-pean mussels, 184–187
 - resistance to, 489
 - M. mactrae*, in Pacific clams, 188
 - M. orientalis*
 - introduction to France and US with Pa-cific oyster, 337, 495
 - in Pacific edible molluscs, 187–188
 - M. porrecta*, in mussels, Gulf of Mexico, 188
 - Mytilus*, infection by protozoans, 113–114
 - M. californianus*, infection by *Mytilicola ori-entalis*, 187, 188
 - M. edulis* (blue mussels)
 - hemocytic neoplasia, malignant, 220–221, 222
 - pollution and, 425
 - infection by
 - copepods *Mytilicola* spp., 184, 185–188, 489
 - protozoans, 109, 113
 - trematode larvae
 - pearl-producing, 151, 239
 - Proctoeces maculatus*, 152
 - rickettsias, 61
 - mass mortalities, unknown etiology, 362–363
 - pollution effects
 - gametogenic activity and, 432
 - mutation rate and, 427
 - nonneoplastic granulocytomas and, 425–426
 - parasitism increase, 434
 - M. galloprovincialis*, infection by
 - copepod *Mytilicola intestinalis*, 184–185
 - fungus in byssus, 73–74
 - Myzobdella lugubris*, in blue crab, 167–168
- N
- Nacrezation, pearl production in bivalve molluscs and, 253
 - Nassarius obsoletus*, infection by trematode, human dermatitis and, 155, 463–464
 - Nematopsis* (gregarine), in brown shrimp digestive tract, 123
 - N. ostrearum*, in oysters, 112
 - N. panopei*, in crabs, 128
 - Nemerteans, on crab and lobster eggs, 166–167
 - Neoplasms
 - in crustaceans, 226–228
 - pollution role, 426
 - in molluscs
 - benign in bivalves, 223–225
 - malignant in bivalves
 - gonadal, 223, 423–425

- hemocytic, 16–17, 211–226, 363–364, 399
 - pollution role, 423–426
 - terminology, 209–210
 - Nephrops*, parasitization by copepods, 197
 - N. norvegicus*, infection by copepod, 197
 - helminths
 - Histiobdella homari*, 168
 - Stichocotyle nephropis*, 164
 - protozoans, 129
 - Neptunus sanguinolentus*, parasitization by copepod, 197
 - Nicthoe* spp. (copepods), parasitizing lobsters, 197
 - Nitzschia pungens*, amnesic shellfish poisoning induction, 474–475
 - Norwalk virus, in molluscs, gastroenteritis outbreaks and, 471
 - Nosema dollfusi*, in trematode parasitizing American oyster, 150
 - N. legeri*, in trematode parasitizing *Donax vittatus*, 154
 - N. ormierei*, in acetosporan parasitizing flat oyster, 492
 - N. (Pleistophora) spelotremae*, in trematode parasitizing green crab, 163
 - Notostomobdella cyclostoma*, on Alaskan king crab, 168
- O**
- Octopus* spp., vibriosis, 47–48
 - O. vulgaris*, infection by
 - cestodes, 158
 - iridovirus, 10, 18
 - Odostomia* (snails), ectoparasitic
 - on oysters, 236–237
 - on scallops, 237–238
 - Opecoeloides fimbriatus*, metacercariae in penaeids, 164
 - Orange sickness, trematode-induced in mussels, 151–152
 - Ostracoblabe implexa*, shell disease induction in oysters, 73, 85, 369
 - Ostrea edulis* (flat oyster)
 - abnormalities
 - ectoparasitic *Odostomia* and, 237
 - shell malformation, 238
 - bacterial diseases
 - Pseudomonas* sp. outbreak, 43
 - rickettsias and, 61
 - fungal shell disease, 72–73, 85
 - mortalities, 366, 368–369, 492
 - hemocytic neoplasia, malignant, 219
 - pollution effects
 - reproductive suppression by oil spill, 418
 - protozoan parasites
 - Bonamia ostreae*, 96, 110–111, 317, 335, 370
 - economic losses in culture, 449–450
 - Haplosporidium armoricum*, 105
 - Marteilia refringens*, 95, 108–109, 316, 317, 369–370
 - economic losses in culture, 449–450
 - hyperparasitization by *Nosema ormierei*, 492
 - O. equestris*, infection by *Perkinsus marinus*, 108
 - O. frons*, infection by *Perkinsus marinus*, 108
 - O. lurida* (Olympia oyster)
 - hemocytic neoplasia, malignant, 219
 - pollution and, 425
 - infection by
 - copepod *Mytilicola orientalis*, 187
 - protozoans
 - Haplosporidium*-like parasites, 105
 - Hexamita nelsoni*, 113
 - O. lutaria* (New Zealand oyster), infection by trematode *Bucephalus longicornutus*, 149–150
 - O. vitrificata*, infection by pinnotherid crab, 189–190
- P**
- Pagurus* spp. (hermit crab), infection by *Peltogaster paguri*, 193
 - Palaemonetes pugio*, black gill syndrome, induction by chemicals, 429
 - Palaemon serratus* (prawn)
 - brown spot disease, bacterial, 51, 314
 - mycosis, 314
 - parasitization by isopod, 196
 - Palinurus vulgaris*, fungal infection, 84
 - Pandalus borealis* (Atlantic shrimp), infection by
 - isopod *Hemiarthrus abdominalis*, 196

- Pandalus borealis* (Atlantic shrimp), infection by (*cont.*)
Microsporidium sp., 122
Hysterothylacium larvae, 166
P. jordani (Pacific shrimp), infection by
Thelohania butleri, 122
Panope abrupta, infection by flagellate *Isonema* sp., 115
Panopeus herbstii (mud crab), infection by protozoans, 112, 128
P. marmoratus, infection by gregarine, 128
Panulirus argus (West Indian spiny lobster)
 humoral responses to infection, 274–276
 infection by protozoan, 129
P. interruptus (California spiny lobster),
 natural agglutinins, 277–278
Parachristianella, larvae in bivalve molluscs, 158
Paragonimus spp., in crabs, human lung infection, 465
Paralithodes camtschatica (king crab)
 bacterial rust disease, 48–49
 infection by
Briarosaccus callosus rootlets, 192
 microsporean, 124
Paralithodes platypus (blue king crab),
 infections
 bacterial rust disease, 48–49
Briarosaccus callosus rootlets, 192–193
 microsporean, 124
 rickettsial, 61
 Paralytic shellfish poisoning, dinoflagellate-induced, 473–476
Paramoeba perniciosus
 in blue crab, 124–126, 272, 393, 451
 in lobster, 126
 in rock crab, 126
Paranophrys (ciliate), in crustacean hemolymph, 126–127
Paranophrys maggii, crab hemolymph reaction to, 276–277, 278
Parorchis acanthus, in oyster drill, 154–155
 Pearl production, trematode-induced in bivalves, 150–151, 238–239
 nacreization and, 253
Pecten maximus, rickettsial infection, 61
Peltogaster paguri, in hermit crabs, hyperparasitization by isopod, 193
Penaeopsis akayebi (prawn), parasitization by isopod, 196
Penaeus, diseases in culture
 epizootics, 314, 317–319
 importance assessment, 321–322
P. aztecus (brown shrimp)
 infectious diseases
 bacterial
 shell diseases, 50, 51
 vibriosis, 57, 318, 460
 cestode *Prochristianella* spp. and, 164
 gregarines in digestive tract, 123
 microsporean-induced cotton shrimp condition, 120–121
 neoplastic and nonneoplastic growths, 227
 pollution and, 426
P. californiensis, infestation by *Leucothrix mucor*, 59
P. duorarum (pink shrimp)
 infectious diseases
Baculovirus penaei and, 21, 27, 29, 30, 34, 318
 latent infection activation by stress, 422
 gregarine in digestive tract, 123
 microsporean-induced cotton shrimp condition, 120
Prochristianella spp. and, 164
 pollution effects
 cadmium-induced gill lesions, 428, 431
 PCB-induced histopathology, 429
P. indicus, viral infection, 30
P. japonicus
 baculoviral infection, 318
 vibriosis, 57
P. marginatus, rickettsial infection, 61
P. merguensis, viral infection in culture, 450
P. monodon (tiger prawn), phycomycete isolation from, 81
P. semisulcatus, viral infection in culture, 450
P. setiferus (white shrimp)
 infectious diseases
 microsporean-induced, 120, 121–122
 phycomycete and, 80
 nonneoplastic growths, 227
 pollution and, 426

- P. stylirostris*
blunted head syndrome, 241
infection by
 IHHNV in culture, 450, 494–495
 rickettsias, 61
- P. vannamei*
hematopoietic sarcoma, malignant, 227–228
infection by IHHNV, 450, 494–495
- Penilia avirostris*, population reduction by
 phycomycete, 395
- Perkinsus*, in abalone and clams, 115
- P. marinus*, in oysters
 encapsulation by host, 252
 epizootics
 in Hawaii, 381
 on US east coast, 96, 105, 315
 economic losses in culture, 448–449
 mass mortalities, 338–339, 375–376,
 382–383
 resistance development during,
 265–267, 489
 salinity and, 107, 266, 376
 stock manipulation in culture and,
 317, 343–344, 346, 347
 temperature and, 106–107, 266,
 383, 448–449
 life cycle, 106, 108
 phagocytosis by host hemocytes, 251
 taxonomy, 76, 105–106, 108
- Pesticides
 effects on oysters, 429
 fungal infection and, 434
 in seafood, public health and, 478–479
- Petroleum, effects on
 bivalve molluscs, 428, 431–432
 lobsters, 428–429
- Petrolisthes armatus*, infection by micro-
 sporean, 124
- P. japonicus*, parasitization by isopods, 194
- Phage clearance, in crabs, 279–280
- Phagocytosis, by hemocytes
 in bivalve molluscs, 251, 262, 263
 copper ion effects, 433
 in crustaceans, 268–271, 285
- Philophthalmus* spp., in marine snails,
 human infection, 467
- Pinctada margaritifera* (pearl oyster), benign
 tumors, 224
- Pinctada martensii* (pearl oyster), infection
 by bucephalid trematodes, 150–151
- Pinnotheres maculatus*, in bivalve molluscs,
 189
- P. ostreum*, in American oyster, 189
- P. pisum* (pea crab)
 infection by phycmycetes, 81–82
 as parasite in bivalve molluscs, 189
- Pinnotherid crabs, in bivalve molluscs,
 188–190
- Placocopecten magellanicus* (sea scallop)
 infection by rickettsias, 61, 363
 mass mortalities, unknown etiology, 363
 shell deformity, coelenterate growth
 and, 236, 237
- Plectospira dubia*, in crustacean eggs, 82
- Pleistophora*, cotton shrimp condition in-
 duction, 119, 121
- P. cargo*, in blue crab muscles, 124
- Pollution
 crustacean responses, 416
 latent infection activation, 422
 shell disease, 419–421
 tumor-like growth, 426
 disease resistance and, 433–434
 lesion induction by chemicals, 427–432
 mollusc responses, 415–416
 cellular, 432
 genetic abnormalities, 426–427
 growth retardation, 417–418
 latent infection activation, 422–423
 neoplasia, 423–426
 oil spill effects, 418–419
 shell abnormalities, 426
- Polydora* (annelid), shell abnormality in-
 duction in oysters, 162, 238, 417
 nacrezation and, 253
- Polymorphus major*, larvae in rock crab, 165
- Porospora* spp., in lobsters, 129
- Portunio conformis*, parasitizing shore
 crab, 194
- Portunus pelagicus*, infection by *Sacculina*
 granifera rootlets, 191–192
- Postmonorchis donacis*, larvae in clams, 153
- Precipitins, response to infection in lob-
 sters, 278–279
- Probopyrus* spp., parasitizing shrimp, 196
- Prochristianella* spp., larvae in shrimp, 164
- Proctoeces*, metacercariae in oysters, 150

- P. maculatus*, progenetic stages in mussels, 152
- Protonyaulax*, paralytic shellfish poisoning induction, 474, 476
- Prototheca lacinata*, infection by *Eche-neibothrium*, 157
- P. staminea*, infection by
cestodes *Echeineibothrium*, 157
coccidian *Pseudoklossia*, 114
- Protozoans, *see also specific protozoans*
in crustaceans, 117–130
 crab diseases, 123–129
 lobster diseases, 128, 129–130
 shrimp diseases, 118–119 (table), 120–123
in molluscs, 95–117
 clam diseases, 114–115
 mussel diseases, 113–114
 oyster diseases, 95–113
nematode-hyperparasitizing, in clams, 160–161
trematode-hyperparasitizing
 in clams, 153–154
 in crabs, 163
 in oysters, 149–150
- Pseudocarcinonemertes homari*, on lobster eggs, 167
- Pseudomonas*, molluscan disease induction, 43, 44, 46, 47
- P. enalia*, mass mortalities in oysters and, 44
- P. perolens*, bactericidal activity induction in lobsters, 284–285
- Pteriidae* (pearl oysters), larval trematode invasion, 150–151
- Public health
 human diseases passively transmitted by shellfish, 468–480
 microbial, 469–473
 natural biotoxins (shellfish poisoning), 473–477
shellfish pathogens
 capable of infecting people and animals, 466–468
 human infection-inducing
 trematodes, 463–466
 Vibrio parahaemolyticus, 459–463, 471, 480
shellfish poisoning
 diatom-induced, amnesic. 474–475
 dinoflagellate-induced
 diarrheic, 474–476
 neurotoxic, 474–475
 paralytic, 473–476
 toxic chemicals of industrial origin in seafood, 477–480
- Purpura (Thais) lapillus*, infection by trematode, 155
- Pythium thalassium*, in crustacean eggs, 82
- R**
- Retrovirus, in soft-shell clams, 16–17, 217
- Rhabdochona uca*, larvae in crab *Uca mani*, 165
- Rickettsial infections, molluscs and crustaceans, 60–62
 sea scallop mortalities and, 363
- Rust disease, bacterial, in king crab, 48–49
- S**
- Saccostrea commercialis* (Sydney rock oyster)
 benign tumors, 224
 infection by *Marteilia sydneyi*, 95, 111, 381
 winter mortality, 380–381
- Sacculina carcini*, rootlets in crabs, 191
- S. granifera*, in Australian sand crab, 191–192
- Sarcomatoid proliferative disease, *see* Hemocytic neoplasia
- Saxidomus giganteus* (butter clam)
 hemagglutinins for human A antigen, 253
 polypoid tumor, 225
- Sepia officinalis*
 benign tumors, 225
 iridovirus infection, 10
- Shell disease
 bacterial
 in crabs, 48–50
 in lobsters, 51–53, 305–306, 319–320
 in prawns, 51
 in shrimp, 50–51
 fungal, in oysters, 72–73, 85, 366–369, 492
 pollution-induced, in crustaceans, 419–421

- Shellfish diseases, economic effects, 443–453
 consumer responses, 445–447
 direct, 443
 indirect, 444
 losses
 in aquaculture, 447, 450
 in natural populations, 450–452
 producer responses, 444–445
Sirolpidium zoophthorum (phycomycete), in oyster and clam larvae, 74
 epizootics in hatcheries, 312–313
Spisula solidissima (surf clam), infection by nematode *Sulcascaris sulcata*, 159–161
 hyperparasitization by protozoans, 160–161
 SSO (seaside organism), *see* *Haplosporidium costale*
Steinhausia spp., in mussels, 113–114
Stichocotyle nephropis, in lobster, 164
 Stress, environmental
 contaminant, *see* Pollution
 effects on oyster diseases
 bacterial infections and, 44–45
 Haplosporidium nelsoni-induced, 98–100, 260–262
 mass mortalities and, 371, 373, 380, 382
 gaffkemia in lobsters and, 286
 in shellfish culture, 330–332
Sulcascaris sulcata, larvae in surf clam, 159–160
 hyperparasitization by protozoan, 160
S. sulcata, larvae in surf clam, hyperparasitization by protozoan, 160–161, 492
 Red tail disease, in lobsters, *see* Gaffkemia
Renicola roscovita, larvae in periwinkle, 154
 Resistance to infection, 489–490; *see also* Internal defense mechanisms
 pollution effect, 433–434
Synophrya (ciliate), in crabs, 127
- T**
- Tapes semidecussata* (Manila clam), infection by *Tylocephalum*, 157
Tellina tenuis, rickettsial infection, 61
Tellina virus, isolation from molluscs, 19
Terebrospira lenticularis, in shrimps, 123
Teredo spp., infection by *Haplosporidium* sp., 115
Tetrarhynchus, larvae in cephalopods, 158
Thais haemostoma (oyster drill), biological control by larval trematodes, 154
Thanatosstrea polymorpha (protozoan), *see* Iridoviruses, in oysters, 316
Thelohania butleri, in pandalid shrimp muscles, 122
T. duorara, in penaeid shrimp muscles, 120
T. maenadis, in crab muscles, 124
T. penaei, *see* *Agmasoma penaei*
T. petrolisthis, in crab muscles, 124
Thraustochytrium inglei, in oysters, 75
Tiostrea lutaria, infection by *Bonamia* sp., 111
Tresus capax, infection by haplosporidian, 114
T. nuttalli
 benign tumors, 225
 infection by larval cestodes, 157–158
 Tributyl tin, shell malformation induction, Pacific oyster, 427
Trichomaris invadens (ascomycete), in Tanner crab, 76, 79, 83–84, 451–452
Tritonia diomedea, fungal infection, 75
Trochicola enterica (copepod), in gastropods, 188
Tylocephalum, larval, in bivalve molluscs, 155–157
- U**
- Uca mani*, infection by larval *Rhabdochona uca*, 165
Unicaleuthes (copepod), on exoskeleton of American lobster, 197
Urosalpinx cinerea (oyster drill), control by phycomycete, 74
 by trematode, 154
Urosporidium constantiae, in trematode parasitizing *Ostrea lutaria*, 149–150
U. crescens, in trematode parasitizing blue crab, 163
U. pelseeneeri, in trematode parasitizing *Donax trunculus*, 153
Urosporidium spisuli, in nematode parasitizing *Spisula solidissima*, 160–161, 492

V

- Vaccination, lobster protection against
gaffkemia, 56, 287, 333
- Vibrio alginolyticus*, in penaeid shrimp,
318
- V. cholerae*, in shellfish, cholera outbreaks
and, 462-463, 471-472
- V. parahaemolyticus*
in blue crab, 58, 349, 460
human gastroenteritis outbreaks and,
459-462, 471, 480
shedding operations and, 307
in brown shrimp, 57, 318, 460
in lobsters, 58, 460
aquarium-held, 306
in oysters, 44-45
- V. tubiashii*, in molluscan hatchery, 312
- V. vulnificus*
in blue crabs held for shedding, 307
as human pathogen, 462
- Vibriosis, mortality induction
in crustaceans, 57-58, 59-60
impounded populations, 306-307
in molluscs, 42-48
antibiotic effects in hatcheries, 312
- Viral diseases, *see also specific viruses*
in crustaceans, 20-34
characteristics, 21-26 (table)
examples, 30-31, 450
generalizations, 32-33
methods of detection, 27-29
shrimp mortalities in culture, 31-32,
450, 494-495
in cultured shellfish, importance, 323-
324
human, transmitted by shellfish, 470-471
in molluscs, 10-20, 34
characteristics, 12-15 (table)
examples, 10-11, 16-19
gill disease in oysters, 11, 16-17, 316,
339-340, 369-370

SH
171 Principal diseases of marine
.S53 fish and shellfish
1989
v. 2

Library
NOAA, National Marine Fisheries Service
SWFC Honolulu Laboratory F/SWC2
2570 Dole Street
Honolulu, HI 96822-2396

Principal Diseases of Marine Fish and Shellfish

Volume 2

Diseases of Marine Shellfish

Second Edition

Carl J. Sindermann

Oxford Laboratory, Northeast Fisheries Center, National Marine
Fisheries Service, Oxford, Maryland

From a Review of the First Edition:

"A timely book, well written, profusely illustrated and documented by an author who has had many years of experience in the field of fish pathology."

—AMERICAN SCIENTIST

This second edition has been expanded to two volumes, the second of which focuses on marine shellfish. Volume 2 reviews the important diseases of wild, captive, or cultivated shellfish species, shellfish immunology, the effects of disease on populations, and public health aspects of shellfish diseases. Fishery scientists and managers, marine biologists, marine ecologists, and marine aquaculturists will find this volume indispensable.